EVALUATION OF RECIRCULATING HYDROPONIC SYSTEMS FOR *EUCALYPTUS* CUTTING PRODUCTION

BY

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PREFACE

The experimental work described in this thesis was conducted at the Trahar Technology Centre, Mondi Forests and the Research Centre for Plant Growth and Development, University of Natal, Pietermaritzburg, from 1998 to 2002 under the supervision of Professor J. Van Staden and the co-supervision of Miss Felicity Blakeway from Mondi Forests, Technical Department.

The results have not been submitted in any other form to another University and except where the work of others is acknowledged in the text, are the results of my own investigation.

GIORDANO DA COSTA ALPOIM

MAY 2002

We certify that the above statement is correct.

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CONFERENCE CONTRIBUTIONS

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ABSTRACT

In South Africa, *Eucalyptus grandis* and hybrids of this genus are planted over 196000 ha of Mondi Forests land. The temperate and cold tolerant species (*E. grandis*, and *E. grandis* X *E. nitens*) occupy 142000 ha and the subtropical species (*E. grandis, E. grandis* X *E. camaldulensis* and *E. grandis* X *E. urophylla*) occupy a further 54000 ha.

In Southern Africa, until 1998, there had not been any significant research undertaken into the development and application of hydroponics to cutting production in nurseries. Since then, research in Mondi Forests has focused on the evaluation of recirculating hydroponic systems for cutting production of the genus *Eucalyptus*.

South American forestry companies have achieved noteworthy success in improving the rooting of cuttings of selected clonal *Eucalyptus* harvested from hydroponic gardens. Verifiable increases of up to 300% in rooting have been achieved. (JANSE, 2001 pers. comm.[1]). Towards this aim, protocols for the production of *Eucalyptus* cuttings in hydroponic systems were established, and implemented in a commercial hydroponic production system.

Seven nutrient solutions with different concentrations of macro- and micro-nutrients were tested on three different substrates, *viz* perlite, sand and 6.2 mm dolomitic gravel. A pure nutrient film system (NFT) was also tested. Nutrients (1.2 -1.5 mS/cm) applied cyclically over a seven day period, followed by two days of leaching with clean water, were found to be suitable for cutting production. Excessive nutrient supply resulted in soft foliage, susceptibility to powdery mildew (*Oidium eucalypti*) and *Botrytis* infection. Foliar analyses showed that macro-element levels in leaves were similar to those of soil-derived plants, whilst micro-element levels were generally higher in the 'hydro-ramets.' Most significant were the higher concentrations of boron and calcium.

Sand and perlite had the best water holding capacities, requiring hydration every 96 hours, whilst gravel sustained plants for up to 48 hours without any visible stress. The modified NFT unit, however, was the most efficient system in terms of managing cutting production.

[1]. Dr. B. Janse. Mondi Forests. PO Box 39 Pietermaritzburg 3200. South Africa.

Some post-establishment mortalities occurred across all treatments and were attributed to pathological attack by *Pythium* sp.

Light source and intensity were critical. Initial low light levels (615 Lux) did not result in optimum growth and plants showed signs of being stressed, thus contributing to an increase in powdery mildew infection. Plant growth improved with increasing light levels (> 3000 Lux).

Following this research, operational systems were developed. From first harvests, rooting of cuttings from the hydroponically-grown parent material were higher than those from field hedges. Mean rooting from the best performing nutrient and substrate combination was 67%, compared to 45% obtained from the field hedge. In addition, the hydroponic system was found to offer other advantages: 1) hydroponic hedges are less costly to maintain than field hedges; 2) hydroponic systems offer greater control of nutrients than can be achieved in field hedges; 3) hydroponically-grown hedge plants can be better shielded from environmental variables which impact on the productivity of field hedge plants; 4) the threat on the environment in terms of water use and effluent disposal is alleviated to a large degree.

CONTENTS

LIST OF ABBREVIATIONS

µmho	micromho
AD	'Anno Domini'
AI	Active ingredient
ARC	Agricultural Research Council
ATP	Adenosine triphosphate
BC	Before Christ
BI	Biomass index
BOF	Biological oxygen demand
CEC	Cation exchange capacity
CF	Conductivity factor
COF	Chemical oxygen demand
CV	Coefficient of variation
DFH	Deep Flow Hydroponics
DM	Dry matter
DRIS	Diagnostic & recommendation integrated system
DW	Dry weight
DWAF	Department of Water Affairs and Forestry
E. GxN	*Eucalyptus grandis x Eucalyptus nitens*
EC	Electrical conductivity
EL	Expanding leaves
ERD	Effective rooting depth
FABI	Forestry & Agricultural Biotechnology Institute
FAD	Flavin adenine dinucleotide
GDH	Glutamate de-hydrogenase
GIS	Geographical information system
GS/GOGAT	Glutamine Synthase
IAA	Indole-3-acetic acid
ICFR	Institute for Commercial Forestry Research
K*m*	Michaelis constant
LSA	Leaf specific area
MAP	Mean annual precipitation
MASL	Mean above sea level (Altitude)
MAT	Mean annual temperature
mmho	millimho

mS/cm	milliSiemen/centimetre
NFT	Nutrient Film Technique
PAR	Photosynthetic active radiation
pKa	Acid dissociation constant
RAR	Relative addition rate
RCD	Root collar diameter (mm)
R:FR	Red : Far Red ratio
RGR	Relative growth rate
RH	Relative humidity (%)
SA	Shoot apex
UC	Uniformity coefficient
SL	Supplementary lighting
VA	Versicular arbuscular (Mycorrhizae)
Vmax	Maximum rate of adsorption
YFEL	Youngest fully expanded leaf

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CHAPTER 1

INTRODUCTION

1.1 Understanding eucalypts and their nutritional requirements

The eucalypts as a genus present remarkable diversity in form and phenology, and an equally remarkable range in physical attributes. Native habitats range from very fertile to infertile, and populations derived from such contrasting sites show variations in traits even within a single species. Provenances are known to differ significantly in growth and nutrient acquisition under severely limiting conditions, and in response to additional supply (KRIEDEMANN and CROMER, 1996). Increasing the productivity of eucalypts in production forestry requires the integration of research of both nutritional physiology and genetics. As mechanisms for the efficient utilisation of nutrient elements will be under genetic control, further work to elucidate key nutrient processes involved in growth may offer opportunities for genetic manipulation and for the breeding of physiologically superior clones (GROVE *et al.*, 1996).

Most nurserymen advocate that the rooting potential of genetically improved eucalypt mother plants (stock plants) is strongly linked to their nutrient status. However, achieving and sustaining this optimum nutritional balance is difficult and complex in practice. In order to accurately predetermine the optimum plant nutrition required all year round, to ensure economic levels of rooting, a more controllable environment is essential. The temperate hybrid, *Eucalyptus grandis x E. nitens,* is a typical example of a hybrid with outstanding volume yields in the field and highly sought after fibre properties, yet it is still extremely difficult to vegetatively propagate at economically sustainable levels. Hydroponics may facilitate the uniformity and control in areas such as nutrition, aeration and CO_2 enrichment. At the same time it may be possible to manipulate the system to accurately determine what level of fertilisation will provide the highest rooting and more importantly allow us to practically maintain that level.

1.2 Maximising genetic gains in forest plantations

DE ASSIS (2001) states that the cloning of forest species has shifted from its original focus of producing greater volumes of timber and disease resistance to the industrial requirements of the processors. The fibre properties that positively influence industrial processes and product quality are now strongly considered as areas where clonal production can play a major role. In the era of global market competition, the development of forest plantations must aim to increase industrial competitiveness in distinct market sectors. The modern forestry approach

must be one of producing a raw product in a sustainable manner with a minimum impact on the environment. The vegetative propagation methods utilised must be able to rapidly transform the genetic gains realised through breeding into benefits for the processor. One of the most efficient tools to ensure these objectives is the combination of inter-specific hybridisation and the establishment of clonal plantings derived from superior hybrid individuals.

To exploit the heterogeneous nature of *Eucalyptus* hybrids and benefit from the quick integration of the genetic gains, a large scale clonal propagation system must be available to deploy the material. DE ASSIS (2001) suggests that mass vegetative propagation perfectly complements hybridisation. According to ZOBEL (1992), by capturing the total genetic variance, vegetative propagation allows for the maximum benefits of wood properties and productivity, besides allowing for the production of a more uniform raw material, which from an industrial standpoint benefits the process and product quality.

KRETZSCHMAR and EWALD (1994) state that the vegetative propagation of forest trees that have demonstrated superiority is strongly desired for assembling of clonal populations with improved characteristics obtained through breeding.

1.3 The field clonal hedge - an outdated propagation system

The first vegetative propagules for the establishing of clonal forests were obtained from field plantations (DE ASSIS, 2001). This management strategy required the annual reservation of large areas for the sole purpose of coppice collection. The clonal hedge system, based on the concept of intensive management was introduced sometime later. CARVALHO *et al.* (1991) and HIGASHI *et al.* (2000) reported that despite being a tremendous advance for the mass production of shoots, clonal hedges were complicated to manage and poorly controlled. To meet the demands of clonal forestry, the system required large areas, intensive labour management and large quantities of fertiliser and water. The propagators were inevitably reliant on the prevailing weather, as the system was at the mercy of environmental conditions.

Little has changed in the management of field clonal hedges since their first plantings some twenty years ago and protocols developed then are still generally applied. In order to produce eight million plants annually, the Mondi subtropical eucalypt clonal nursery at Kwambonambi (KwaZulu-Natal, South Africa) has to maintain clonal hedges of some 25 ha. This scale of operation has massive logistical implications and is extremely labour intensive.

In 1993 a decision was made to start production of temperate eucalypt clones at the Mondi Mountain Home Nursery (Hilton, KwaZulu-Natal). Although not as extensive as the subtropical coastal operation, this programme has the added confounding issue of major climatic differences throughout the year. With the onset of winter there is a distinct decrease in the amount of coppice material available for the production of cuttings. Paradoxically, the rooting increases during autumn to early winter when there is the least amount of coppice for sustainable production.

DE ASSIS (2001) supports local experience when stating that in eucalypts, the popular method of rooting stem cuttings has limitations, such as the rapid loss of rooting competence due to ontogenetic ageing, intra-clonal variation resulting from topophysis, and poor quality root systems that negatively affect the genetic expression of superior clones. SCHÖNAU (1981 & 1982), LAMBERT *et al.* (1983), and GROVE (1990) all agree that nutrient concentrations in eucalypt foliage and other components are sensitive to both site fertility and fertiliser addition. The application of nutrient analysis to the identification of nutrient imbalances and for monitoring gains in productivity due to addition of fertiliser, has attracted considerable attention and is a major focus of research in eucalypt nutrition. Unfortunately, the ability to practically interpret such analytical results has its own complexities that tend to confound most nurserymen.

1.4 Hydroponic culture - an effective technology for clonal forestry

The current system of extensive clonal hedges is costly and difficult to maintain. The Brazilians have proven that hydroponics has a major role to play in the future clonal propagation of subtropical hybrid eucalypts such as *E. grandis x E. urophylla* (JANSE, 2001 pers. comm.[1]). Our challenge is to determine whether **all** of the important eucalypts currently planted in South African forests can be propagated from hydroponically sustained mother plants. This technology holds the potential to revolutionise clonal production capacities and improve the overall efficiency of existing nurseries.

The planting of high quality transplants is crucial to achieve high yield and quality of the final products, and the demand for these genetically uniform transplants is expected to increase in the 21st century. Techniques and methods for producing such transplants need to be efficiently developed. Modern propagation technologies often begin with *in vitro* micro-propagation for establishing of virus-free or pathogen-free stock plants, followed by

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propagation *ex vitro* (macro-propagation). The number of plantlets propagated *in vitro* is usually minimised to reduce production costs. Such plantlets are then transferred to sterile soil or hydroponic systems in an arthropod-excluding growth room and grown as stock plants for pathogen-free propagules and transplants (KUBOTA *et al.*, 2001). Although the potential gains of this concept have been widely published by forestry scientists it remains a relatively new and untapped field of propagation.

The use of controlled environments began in the 1980s with an emphasis on the potential benefits to transplants having a high value per unit of production area and a short production time. However, most of these systems have not been adopted by commercial growers because of a general lack of knowledge as how to apply them on a commercial basis. Vegetative propagation has been a significant mode of transplant production (HARTMANN *et al.*, 1990) and the number of species propagated vegetatively has been increasing in commercial transplant production operations.

Hydroponics is the cultivation of plants in a soil free environment (HARRIS, 1987). The emphasis is on an easily modified and accurately controlled nutrient solution that can provide a complete range of nutrients for the plant. The medium used must be neutral and inert and therefore soil as a substrate is unsuitable.

Due to the ever increasing problem of ground water contamination in Europe and the USA, there is a growing awareness of the value of hydroponic systems. In many first world countries laws will soon be introduced requiring the treatment and reuse of all water utilised in a horticultural environment. This in reality is a hydroponic requirement and will lead to more efficient use of fertilisers if strictly controlled (A'BEAR, 1995).

COOPER (1996) has grown a number of different tree species in the Nutrient Film Technique (NFT) and found the stock plants to be of excellent vigour. He considers NFT to be an ideal system for the rooting of cuttings as the micro-environment can be carefully controlled and the efficiency of propagation enhanced. He suggests that the aerial environment can be fully controlled by manipulating light, day and night temperatures, CO_2 content of the air and relative humidity. The use of the NFT for propagation purposes can provide a facility for precise control of the root-zone environment. The nutrient concentration, pH and growth regulator content of the solution at the cut surface, can also be precisely controlled. With this degree of manipulation over both the aerial and root-zone environment, all that is theoretically necessary is to develop the optimum protocols to suit the specific crop.

1.5 Aims and objectives

FETT-NETO *et al.* (2001) describe rooting as an essential step in the vegetative propagation of economically important woody species. Adventitious rooting is a complex process, which is affected by multiple factors including phytohormones, phenolic compounds, nutritional status and genetic characteristics, as well as associated stress responses such as wounding, changes in plant water relations and loss of correlative whole plant influences.

Hydroponics and its suitability to the propagation of exotic forest species has had very little attention outside of South America. The aim of this study is to determine the feasibility of utilising **recirculating hydroponic systems** to improve the overall rooting efficiency of cuttings harvested from clonal *Eucalyptus* hedge plants, and the ability of hydroponic parent plants to supply higher numbers of rooted cuttings than field hedges can.

Major objectives are to focus on the role of nutrition and attempt to identify those nutrients that strongly correlate with the rooting of eucalypts. The impact of pathogens on ramet production also has to be monitored as disease is the greatest threat to the survival of stock plants in soilless culture. In order to fully understand the implications of hydroponic technology on the clonal propagation of eucalypts, it is important to discuss the role of nutrition in the genus and consequently, Sections 2.1 to 2.6 explore the role of nutrition in eucalypts. Sections 2.7 to 2.10 focus more on the concept of hydroponics and its future in the propagation of clonal eucalypts.

CHAPTER 2

LITERATURE REVIEW

2.1 *Eucalyptus* plantations in South Africa

2.1.1 Natural distribution of eucalypts

ELDRIDGE *et al.* (1993) and JUDD *et al.* (1996) state that the genus *Eucalyptus* L'Hér is large, comprising at least 500 species, most of which are endemic to Australia but some occur naturally in Papua New Guinea, Indonesia and the Philippines. Although the eucalypts reach their greatest diversity in the moist south-east and south-west corners of Australia they grow throughout the continent, and the hardiest species survive even the driest areas (200 mm annual rainfall). While soil fertility is an important factor, the major determinant of both the distribution and productivity of eucalypt species in Australia is the availability of water determined by climate, topography (aspect, slope) and soil (structure, organic content, depth, proportion of rock, stone, gravel).

The wide range of ecological adaptability and genetic diversity of *Eucalytpus* has been reflected in the successful use of eucalypts in plantations around the world. Many of the eucalypts grow extremely fast even on relatively harsh nutrient-poor sites. Their superiority over other tree species has made them a first choice planting in many parts of the tropics and subtropics.

2.1.2 Distribution of eucalypts in South Africa

Eucalypts are planted commercially between latitudes 23^0 and 34^0 where the mean annual precipitation (MAP) exceeds 850 mm, and may even exceed 2000 mm. The lower rainfall limit tolerated by eucalypts in South Africa rises with mean annual temperature (MAT), which in turn is influenced by latitude and longitude. The seasonal rainfall pattern varies across the country all year round, from winter to summer, while droughts may be prolonged and severe. The wide and scattered distribution of forestry in South Africa therefore, covers a considerable variation in climate, especially temperature (HERBERT, 1996). This aspect of site diversity is the major reason for working with a range of species in the timber industry (WEBB *et al.*, 1980, SCHÖNAU, 1991).

HERBERT (1996) states that the fertility of soils in South Africa is not well documented. Acidic and highly leached subsoils are common to forestry sites, while topsoil organic carbon and exchangeable calcium and magnesium are highly variable. Exchangeable cations tend to increase with clay content, and vary considerably depending on parent material.

2.1.3 Site requirements of eucalypts

Traditionally, eucalypt plantations have been concentrated in the warm-humid to sub-humid regions where the available soil moisture is strongly related to soil type and may therefore, be used as the primary indicator of forestry potential (SCHULZE, 1958). With the increased demand for hardwoods, a range of cooler, hotter and drier regions has been afforested with eucalypts. Both the temperature regime of the site and the soil type become the primary considerations in selecting successful species (SCHÖNAU and GARDNER, 1991). The criteria required by the site to achieve maximum growth of the main eucalypt species are summarised in Table 2.1. The limits set for the mean annual precipitation refer to average temperature requirements for a species, and must be adjusted relative to increases or decreases in the actual mean annual temperature and other factors affecting actual evapo-transpiration.

Table 2.1 Site criteria for the optimum growth of *Eucalyptus* species. ERD - effective rooting depth; MAP - mean annual precipitation; MAT - mean annual temperature

Species	ERD (cm)	MAP (mm)	MAT °C
E. diversicolor	> 90	> 850	(no data)
E. dunnii	> 50	> 850	> 15
E. elata	> 50	> 900	14.5 - 16.5
E. fastigata	> 60	> 900	14 - 16
E. fraxinoides	> 75	> 900	< 16
E. grandis	> 60	> 900	> 16
E. macarthurii	> 40	> 850	< 17.5
E. nitens	> 75	> 900	13 - 15.5
E. saligna	> 55	> 900	> 15.5
E . smithii	> 70	> 900	> 15

(BODEN, 1987)

Due to the drought-prone nature of the climate in South Africa, and the necessity to restrict forestry to those areas where rainfall is consistently high, sites are often selected for the fast growing and demanding eucalypts on the basis of the soil being able to offset these limitations. The soils chosen tend to be deep, well drained, apedal and with relatively thick top soils of at least moderate organic matter-status. Where soils are shallow (30-50 cm ERD) they must be

capable of storing large amounts of accessible soil water, and have a high clay content (HERBERT, 1996).

2.1.4 The potential of the hybrid, *E. grandis x E. nitens* and its value to the South African forestry industry

DE ASSIS (2001) suggests that hybridisation is an option of great value in tree breeding programmes. It facilitates the combining of superior wood properties with tolerance to biotic and abiotic stress and represents a significant source of superior individuals, capable of introducing genetic gains in forest productivity and wood properties. Crossing species of different characteristics allows for the production of complementary fibre properties tailored to meet processor requirements.

DENISON and KIETZKA (1993) noted that inter-specific hybrids are of increasing importance to the South African forest industry. Hybrids have made it possible to extend tree planting to areas traditionally considered off-site for plantation forestry. On marginal sites, the hybrids' growth and survival outperform the pure species and they are consistently more resistant to disease, pests, cold, heat and drought. The wood and growth properties are normally intermediate between the parent species, but superior growth to both parents is common.

Hybrids of subtropical *Eucalyptus* are presently the most prominent in the South African forest industry, with thousands of hectares of selected clonal plantings already established. The most common hybrid combinations are *Eucalyptus grandis* with *Eucalyptus camaldulensis*, *Eucalyptus tereticornis* or *Eucalyptus urophylla*. (WEX and DENISON, 1997). *E. grandis* is frequently favoured in the cross for its excellent growth potential, good form, desirable pulp and superior timber properties. Furthermore, cuttings of some clones of this species have a propensity to root very well. Unfortunately, its susceptibility to cold temperature and fungal diseases in South Africa poses a serious threat to its survival as a pure species in both the subtropical and temperate regions of South Africa (WEX and DENISON, 1997). *E. nitens* is a cold tolerant species that can be planted at higher altitudes than *E. grandis* and occurs naturally between 600 m and 1600 m altitude in Victoria and New South Wales, Australia (STANGER and OSHCROFT, 1993). Under South African conditions, altitudes of between 1300 m and 1500 m are regarded as ideal for *E. nitens*. It is a desirable species for pulpwood production and the wood from mature trees compares favourably with that from the 'Ash' type *Eucalyptus* of Australia. *E. nitens* is planted widely in the Mpumalanga province, along the

Highveld escarpment, where frosts are prevalent throughout the winter months and rainfall is moderate, between 800 mm and 1000 mm. The planting of this valuable species is limited by its susceptibility to leaf spot *(Mycosphaerella mulleriana)*, the incidence of which worsens with increasing temperature, and the scarcity of good quality seed (WEX and DENISON, 1997).

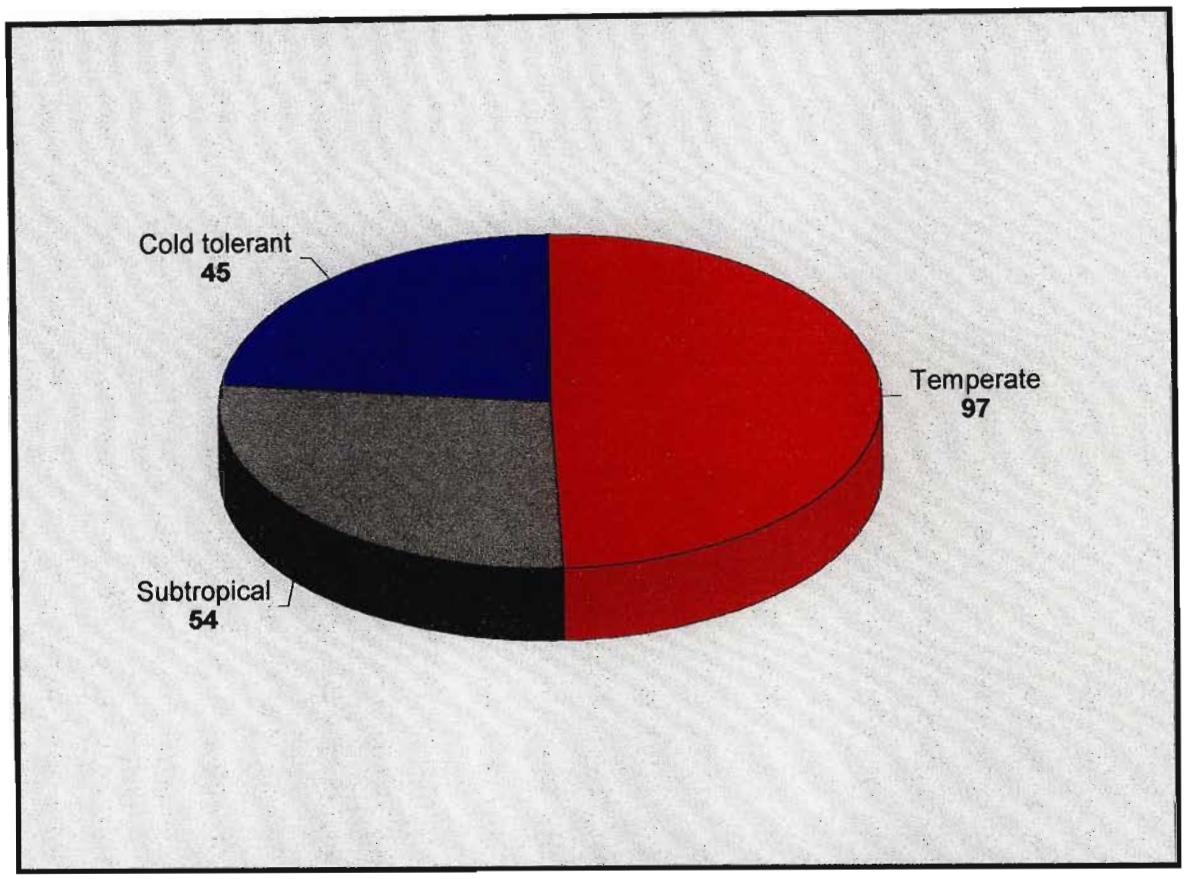
According to WEX and DENISON (1997), the hybrid of *E. grandis* and *E. nitens* has the potential to yield high quality clonal material via the macro-propagation route. Vegetative propagation has allowed the development of clonal forestry on a commercial scale in the subtropical regions of South Africa, and has potential for the cold tolerant and temperate areas of the country.

There are currently 142 000 ha of Mondi Forests land planted to temperate and cold tolerant *Eucalyptus* species (Figure 2.1). The size of these areas in comparison to those planted to the subtropical *Eucalyptus* species and hybrids is indicative of the importance of the 'E. GxN' Hybrid (WEX and DENISON, 1997). KUNZ and DU PLESSIS (2000) mapped Mondi land holdings using the Institute for Commercial Forestry Research (ICFR) GIS system, and determined that 20.4% was suited to the growth of E. GxN hybrids. From numerous clonal trials, the E. GxN hybrid clone has shown itself to be consistently superior in comparison to seedling control lots and other hybrids.

The best gains for E. GxN hybrids have been made on the colder sites of the Mpumalanga Highveld. In addition, improved survival (as compared to the control lots), in extreme frost sites has been observed. Test results indicate that the lower limit of E. GxN clones, with respect to altitude, is approximately 1000 m, nearly 300 m below the lowest limit of *E. nitens*.

The areas available for afforestation with the cold and temperate tolerant eucalypts in South Africa are vast. From the results of trials there is strong evidence to suggest that the E. GxN hybrid will produce substantial gains over the preferred pure commercial species in many of these areas (WEX and DENISON, 1997).

Figure 2.1 Breakdown of afforested area in Mondi Forests by *Eucalyptus* type (temperate, cold tolerant and subtropical) in 000 ha



(WEX and DENISON, 1997)

2.1.5 Site requirements of *Eucalyptus grandis x Eucalyptus nitens*

This hybrid cross most accurately fits into a category between *E. dunnii* and *E. nitens*. The summary below is a composite of our findings in Mondi Forests.

Optimum MAT range: 14.5 °C - 17.5 °C.

Minimum MAP: >830 mm.

Altitude (masl) : 1000 m to 1300 m.

Drought tolerance: Moderate to good - avoid rocky exposed ridges.

Mist: Fairly efficient capture of precipitation.

Hail: Strong apical dominance minimises forking damage to leader.

Snow: Withstands moderate snow falls. Little crown breakage expected.

Frost: Withstands moderate to severe frost. Avoid frost hollows and major drainage lines.

Wind damage: Good resistance, but high chill factors can cause tip and stem die back.

Wind throw: Good stability, unless root deformations from nursery exist or if planted on a shallow site.

Minimum soil depth: ERD= 30-120 cm.

Soil drainage: Moderate to good drainage, avoid gleyed horizons that encourage root pathogens i.e. *Phytophthora* and *Pythium*.

Soil texture: Clay loam texture with good internal drainage. Can grow on all textures found in forestry areas.

Soil structure: Apedal soils most suitable, can tolerate some weak blocky structure.

Stones: Tolerates 30-40% in soil profile.

Lithology: Preference for weathered granite, dolerite and diabase soils and freely draining shale.

Insects: Susceptible (like most *Eucalyptus*) to termite attack on dry sites.

Diseases: Certain clones susceptible to the bacteria *Xanthomonas*. No other major pathogens noted.

Fire: Poor resistance to fire - thin bark.

Comments: a very vigorous species, capable of high yields on good moist sites. Excellent performer on Mpumalanga Highveld. Pulping qualities appear to be '*Grandis*' like. (KUNZ and DU PLESSIS, 2000).

2.2 The evolution of eucalypts

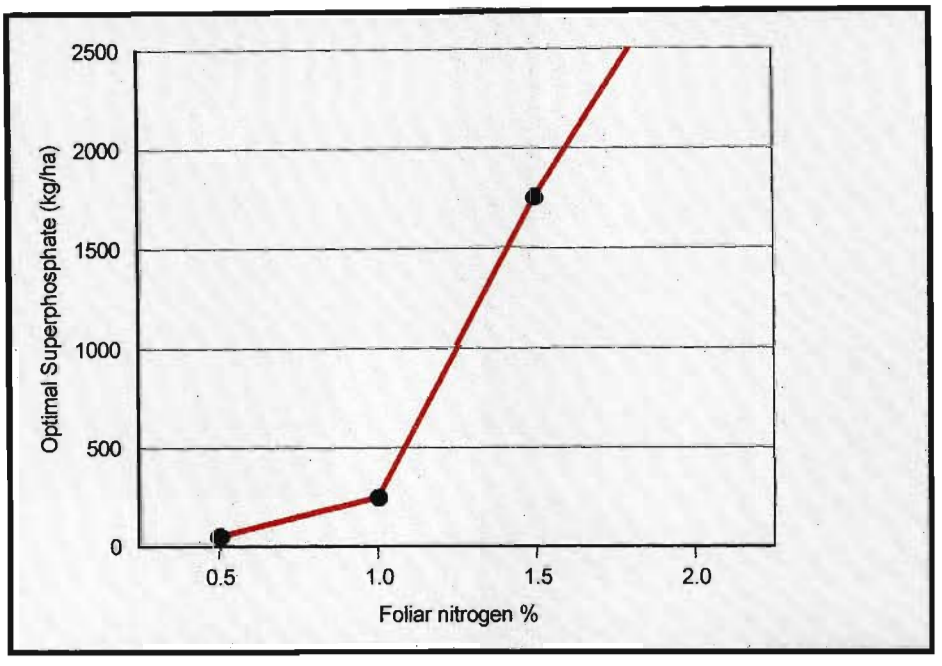
2.2.1 The role of soils

According to SUN *et al.* (2001), soil nutrient supply plays an important role in the distribution of terrestrial plants within the limits defined by temperature and water. Optimal nutrient requirements and tolerance to supply limitation are both important factors in determining the natural distribution of forest trees in relation to soil fertility. SPECHT (1996) states that many of the species of the genus *Eucalyptus* are generalists and show considerable plasticity in growth form. Some may form trees up to 30 m tall, whilst others grow as low shrubs never taller than 2-3 m. Ecotypes of species within the sub-genera *Monocalyptus* and some *Symphyomyrtus*, flourish on soils which have been shown to provide inadequate nutrition to agricultural species. Nutrient conservation strategies such as nutrient recycling, the trapping of nutrients from decomposing litter by rootlets, and the staggered seasonality of foliar shoots and roots, have been identified in a number of species. SPECHT (1996) notes that the *Monocalyptus* species are found on more acidic, low nutrient soils, whereas *Symphyomyrtus* species tend to colonise sites with a greater nutrient availability.

Initially, species of *Eucalyptus* would have evolved at the edge of the rain forests where the dense vegetation became more sparse. Rain forests persisted on well drained soils with greater nutrient availability than in the seasonally waterlogged, lateritic soils. On these soils, seedlings of *Eucalyptus* species with a high nitrate reductase activity in their leaves would have flourished. *E. grandis* W. Hill ex Maiden and *E. saligna* Sm. (both *Symphyomyrtus: Transversaria*) grow at the edges of the rain forests of eastern Australia (SPECHT, 1996)

SPECHT *et al.* (1991) conclude that nutrient conservation strategies have evolved with the speciation of the Eucalypts on nutrient poor soils. Firstly, leaves of seedlings have a high LSA (Leaf specific area) which, with high nitrate reductase activity and photosynthetic potential, enable the seedlings to utilise nitrate ions and other nutrients which are available. The genetically controlled growth potential (the maximum growth of seedlings with increasing additions of phosphatic fertilisers) of eucalypts appears to have decreased progressively in the nutrient gradient from nutrient rich soils to nutrient poor soils.

Figure 2.2 Optimal level of response of four species of *Eucalyptus* to the application of super-phosphate fertiliser, plotted against nitrogen. Concentrations measured in the mature leaves of adult trees



(SPECHT, 1996)

Nutrients released from litter during the season of maximum decomposition are trapped by an interlacing network of fine rootlets with associated rhizosphere organisms. Over-storey eucalypts trap the orthophosphate as long-chain polyphosphates and store these as polyphosphate granules (Figure 2.2). Polyphosphate granules accumulate in both rootlets and associated rhizosphere organisms whenever there is a supply of orthophosphate to an organism in which cell division is minimal; the polyphosphate granules are hydrolysed to orthophosphates as soon as the growth of the organism is initiated (SPECHT, 1996).

SPECHT (1996) indicates that in nutrient-poor soils, polyphosphate synthesis occurs in rootlets during the period of minimum shoot growth and polyphosphates are hydrolysed some weeks later when shoot growth is initiated.

2.2.2 Distribution of eucalypts in Australian landscapes

2.2.2.1 Climate

In broad terms, climate and the availability of water strongly limit the distribution of the eucalypt species. On a continental scale, mallees are confined to drier regions, Ash species dominate wet cool valleys and south facing slopes in south-eastern Australia, while Box, Iron Bark and Red Gum species grow on inland slopes of the Great Divide (ADAMS, 1996). At the landscape

scale, patterns of species distribution and rates of growth are related to land forms and the location of the site in the landscape. Red River gums line the banks of water courses throughout much of the continent. Mountain Ash dominates and grows to great heights in the deep valleys of the Florentine river in Tasmania and in the valleys of the Strzlecki range in Gippsland, Victoria. These soils tend to be phosphate deficient by agricultural standards. Karri (*E. diversicolor*) tend to grow at the bottom of a gully rather than on the sides and aspect or land form plays a major role in species distribution and plant growth. Species distribution and the productivity of a eucalypt forest can be readily related to climate within Australia (ADAMS, 1996).

2.2.2.2 Nutrient cycling and availability

Litter fall, the sloughing of roots, and the death of plants add carbon and nutrients to the surface soil horizons and is the dominant process in the cycling of nitrogen and phosphorus in the forests (Table 2.2). Nutrient cycling aids in the conservation of nutrients. Only in old, 'over mature' forests do outputs of major essential nutrients, especially nitrogen, exceed inputs. Some of the world's most productive forests in the Amazon basin grow on deep sands which have low concentrations of total or extractable phosphorus, and where the phosphorus content of the soil profile is low. The availability of nutrients in these tropical forests is preserved through nutrient cycling and decrease whenever the forest is cleared.

Table 2.2 Concentrations of nitrogen and phosphorus in the youngest, fully expanded leaves of seedlings (<1-year-old) of *E. globulus* supplied with different amounts of nitrogen in a plantation trial. Leaves were assayed in February and April. Growth of seedlings increased with increasing additions of nitrogen up to 160 kg/ha. Seedlings supplied with 160 kg/ha nitrogen were up to three times the height and almost twice the basal area of seedlings to which no nitrogen was added

Amount of nitrogen added (kg / ha)	Nitrogen concentration Feb. (% dry mass)	Nitrogen concentration April (% dry mass)	Phosphorus concentration Feb. (% dry mass)	Phosphorus concentration April (% dry mass)
0	1.66	2.48	0.1	0.17
20	1.85	2.47	0.1	0.16
40	1.97	2.6	0.11	0.16
80	2.17	2.69	0.12	0.17
160	2.20	2.74	0.12	0.17

(ADAMS,1996)

The close relationship between the cycling of nitrogen and phosphorus (Table 2.2) and their availability can be summarised as follows:

- Litter fall is an excellent index of the productivity of mature forests. In Australian eucalypt forests, litter fall and the amount of nitrogen and phosphorus contained within the litter fall, is closely related to the climate. Furthermore, the *turnover* of that organic matter is also related to the climate. The nitrogen content is a product of the amount of nitrogen mineralised in the soil, which in turn meets the demand for nitrogen by the plants.
- The number and size of sinks for orthophosphate ions in the soils, especially old and weathered soils, and the low rates of turnover and uptake of phosphorus (<5 kg/ha/annum), preclude the direct measurement of phosphorus that is mineralised.
- The availability of soil phosphorus is increased by a number of physiological and anatomical features, especially those associated with mycorrhizae or other specialised root structures.
- Phosphorus availability can be modified by plant growth.
- Organic phosphorus may take up one-half or more of the total phosphorus in the soil in eucalypt forests and fractions of organic phosphorus may be related to phosphatase activity, which is essential for the mineralisation of phosphorus esters in the soil.

2.2.2.3 Nutrient cycling

From the nutrient cycling perspective, we can provide a rationale for the relationships observed between the concentration of nutrients in soils and the plants. Much of the relative fertility of forest soils is due to the growth of plants and nutrient cycling, and will be modified by disturbance such as fire (ADAMS, 1996). Higher concentrations in gullies than on ridge tops will be caused, in part, by a long and less disturbed history of plant growth and by geomorphic movement of particulate and soluble nutrients from locations higher in the landscape to those lower down. Availability of nitrogen and phosphorus will be greatest where growth and nutrient turnover is better, and least where growth is poor. While applications of fertiliser may increase the concentrations of some nutrients in foliage in the short term, other limitations to growth and the many, mostly conservative, processes which control the availability and cycling of nutrients will limit long term responses. The hope that a poor forest can be turned into a high quality one by manipulating soil nutrients is a very optimistic one unless all other constraints to growth can be removed. An example occurred in South African fynbos invaded by *Acacia saligna*. Growth of *A. saligna* increased soil concentrations of nitrogen, calcium, magnesium, potassium, manganese and boron without increasing the growth of surrounding indigenous flora (ADAMS, 1996).

2.2.3 Nutritional physiology of eucalypts: uptake, distribution and utilisation

2.2.3.1 Introduction

GROVE *et al.* (1996) state that eucalypts have evolved predominantly on the Australian continent where nutrient availability in most soils is low and limits tree growth. Their survival and growth on these soils depends on mechanisms which enhance nutrient uptake and contribute to efficient use and retention of nutrients within the tree. An important mechanism contributing to efficient uptake by eucalypts is the symbiosis between fine roots and ectomycorrhizal fungi. A broad range of fungal taxa form ectomycorrhizae with the eucalypts, and these fungi are able to occupy different niches in the soil-litter layers and utilise a number of mechanisms in enhancing uptake of nitrogen and phosphorus. The uptake of nutrients that are immobile in the soil, such as phosphorus, is increased through greater exploration of the soil volume by fungal hyphae.

Eucalypts are particularly low in the amount of phosphorus contained in the above ground components, and generally have lower levels of nitrogen than the Northern Hemisphere species. Proportionally more of the dry matter and nutrients are contained within the roots of eucalypts growing in low nutrient or dry environments than on moister, higher nutrient sites. Foliage contains a major proportion of the above ground nitrogen and phosphorus in young trees. Stems and branches of older eucalypts contain most of the tree's nutrients and are major sinks for additional nutrients taken up when the supply in the soil is increased. Strategies which contribute to efficient use of nutrients by eucalypts and to their ability to survive and grow in low nutrient environments include the genetic regulation of maximum growth rates, the capacity to store and reuse nutrients in excess of current requirements for growth, and strong development of biochemical cycling. Nutrient translocation from senescent leaves and from wood in the transition from sapwood to heartwood are the major components of the biochemical cycling of nitrogen, phosphorus and other mobile nutrients. Retranslocation of phloem-immobile nutrients such as calcium is generally a minor component of nutrient transfer within the tree (GROVE *et al.* ,1996).

GROVE *et al.* (1996) indicate that there are a number of gaps in our knowledge of the nutritional physiology of eucalypts. Listed hereunder are a number of the more salient needs:

- A lack of quantitative information on the internal cycling of nutrients.
- Studies to distinguish between potentially mobile and structurally bound forms of nutrients.

- A better understanding of how changes in the supply of nutrients in the soil, either from nutrient applications or other forest disturbances affect uptake, storage and internal cycling of nutrients in the tree.

A better knowledge of eucalypt nutrition will help formulate management options aimed at sustaining and improving the productivity of plantation forests and the propagation of the genus.

2.2.3.2 Nutrient uptake by roots

GROVE *et al.* (1996) state that the nutrient content of soils and reaction of nutrient ions within the soil affect the availability of nutrients to plants, although uptake by roots is largely determined by the absorption capacities of roots and by the mobility of the ions in the soil.

A contributing factor to the low absorption rates reported for seedlings may be the diversion of assimilates to the production of roots that ensure seedling survival. As an example, the early growth of seedlings of *Eucalyptus regnans* F. Muell. is characterised by the initial development of a deep tap root, whilst extensive lateral roots develop later (GROVE *et al.,* 1996).

The absorption capacity of roots can limit the uptake from soil of relatively mobile nutrients (e.g. NO_3^-, SO_4^{2-}, Ca_4^{2-}, Mg^{2+}) which move to the root by mass flow. However, for nutrients which react with minerals and organic matter in the soil and are less mobile (e.g. $H_2PO_4^-$, NH_4^+, Zn^{2+}, Cu^{2+}) diffusion is the rate limiting step. The low specific absorption capacities of plants growing in infertile environments are thought to have arisen because diffusion of nutrients in soil is the primary factor limiting absorption and there is little selective pressure for high absorption capacities. Concentrations of immobile ions in the soil solution are affected by the amount of clay and organic matter, while phosphate also reacts strongly with the surfaces of iron and aluminium oxides. For these ions, depletion zones can develop around roots over short periods of time. In low nutrient environments, absorption of these nutrients is determined by rooting density and other properties of root surfaces (GROVE *et al.,* 1996).

Roots are most abundant in soils where organic matter and nutrients are concentrated. GROVE *et al.* (1996) estimate a rooting density (root length per unit volume of soil, L_V) of 7 cm/cm^3 in the top 10 cm of soil in a forest of *E. marginata* Donn ex Sm., and an L_V of 1-2 cm/cm^3 for surface roots in *E. regnans* forest. In two stands of *E. grandis* W.Hill ex Maiden, irrigated with effluent water, L_v values of 1-2 cm/cm^3 were recorded. These low root densities

may have been as a result of a high nutrient supply. The root densities of eucalypts are well below those found for cereals and grasses and the volume of soil reached by the fine root hairs is relatively small.

The effective root length is generally much greater for species with fine roots than for those with coarse roots. Many eucalypt species have fine roots and depend on root hairs to increase the volume of soil from which nutrients can be absorbed. Auto-radiographic studies have shown that the zone of depletion for phosphorus and zinc in strongly fixing soils corresponds with the volume occupied by root hairs. With eucalypts, root hairs are sloughed off following the development of ectomycorrhizae. Fine root turnover further enhances the absorption of water and poorly mobile nutrients by trees in infertile soils. Frequent sloughing of roots and growth of new roots will maintain a small, fine root biomass and minimise respiratory losses from ineffective roots (GROVE *et al.,* 1996).

2.2.3.3 Mycorrhizae

There are two main types of mycorrhizae:

- **Vesicular-arbuscular (VA)** mycorrhizae in which the fungal penetration of the root and the formation of intracellular structures has little effect on the external appearance of fine roots.
- **Ectomycorrhizae** where fungal tissue forms a sheath around the fine root, resulting in a thickened lateral root with a distinctive pinnate habit (CHILVERS and PRYOR, 1965).

Although ectomycorrhizae are predominant in eucalypts, VA mycorrhizae can develop in seedlings but are rare in older trees. The ability to form associations with VA-mycorrhizal fungi may also help seedlings become established on sites where innoculum of ectomycorrhizal fungi is limited or absent (GROVE *et al.,* 1996). Eucalypt mycorrhizae are formed by a wide range of fungal taxa. The *Cortinarius* species show a preferential development in the litter layer while species in the genus *Hysterangium* occur only in mineral soils (MALAJCZUK *et al.*, 1987). Studies have demonstrated greater phosphatase activity of *Cortinarius* mycorrhizal roots than those formed by *Hysterangium* species.

Mycorrhizae enhance the uptake of phosphorus and other ions of low mobility in soil, including Zn^{2+} and NH_4^+. Phosphorus and nitrogen are the elements that most often limit growth of eucalypt forests, and mycorrhizae play a crucial role in their acquisition. Mechanisms for nutrient uptake appear to be similar for both VA and ectomycorrhizae (GROVE *et al.,* 1996). The uptake of phosphorus is enhanced by increased exploration of the soil by the root fungus

association. External hyphae extending from the root absorb phosphorus from a much larger volume of soil than is possible with uninfected root systems. Uptake of phosphorus is an active metabolic process and is similar both at the root surface and fungal hyphae. Mycorrhizae may enhance the uptake of phosphorus from the soil by absorbing phosphorus from sources which cannot be utilised by non-mycorrhizal roots or by absorbing phosphorus at lower concentrations in the soil solution than non-mycorrhizal roots. The production of oxalic acid by mycorrhizal fungi and roots may also be important in increasing phosphorus uptake by combining with calcium ions or releasing phosphate adsorbed on iron and aluminium oxides in the soil (GROVE *et al.*, 1996).

GROVE *et al.* (1996) observed that mycorrhizal *E. pyrocarpa* LAS Johnson and Blaxell inoculated with specific isolates of ectomycorrhizal fungi redistributed a greater proportion of absorbed phosphorus from fine roots to shoots than non-mycorrhizal plants when the seedlings were starved of phosphorus (Table 2.3) .

Table 2.3 The effect of inoculation with *Protubera canescens* on growth and distribution of phosphorus in *E. pyrocarpa*. Plants were grown in leached white sand, in free draining pots and were continuously supplied with nutrients. At 18 weeks, phosphorus was leached from each pot and no further phosphorus applied. Values within columns followed by the same letter are not significantly different (P <0.10)

Inoculation treatment	Harvest (weeks)	Dry wt. of whole plant (g)	Shoot P content (mg/plant)	Total roots P content (mg/plant)	Fine roots P content (mg/plant)
Uninoculated	18	7.63a	3.52a	1.41a	0.78b
Uninoculated	23	14.88b	3.70ab	1.51a	0.57ab
Inoculated with ***Protubera canescens***	18	8.57a	3.53a	1.71a	0.78b
Inoculated with ***Protubera canescens***	23	16.71c	4.37b	1.32a	0.25a

(GROVE *et al.*, 1996)

HEINRICH and PATRICK (1986) suggest that there may be no response in shoot growth to increased uptake and translocation of phosphorus until significant amounts of phosphorus are transported to the shoot.

Ectomycorrhizae play an important role in the nitrogen nutrition of trees. In many eucalypt forest soils, ammonium is the dominant inorganic form of nitrogen. (ADAMS and ATTIWELL, 1986). Most ectomycorrhizal fungi in pure culture utilise, and prefer, ammonium to nitrate. Much of the biomass of ectomycorrhizal fungi associated with tree roots is in the soil layers where nitrogen mineralisation occurs. This fungal mycelium is better able to compete for NH_4^+ than tree roots because it is strategically placed. Saprophytic microbes will compete strongly where NH_4^+ is in low concentrations in the soil, and trees will benefit where NH_4^+ is captured

and sequestered in mycorrhizal hyphae. Although most of the nitrogen taken up by ectomycorrhizae originates in inorganic forms, some ectomycorrhizal fungae can produce enzymes which release nitrogen and hence may obtain nitrogen from organic sources in the soil. The nitrogen absorbed by hyphae is rapidly assimilated into a range of amino compounds, particularly glutamine. Ammonium assimilation potentially involves both the glutamine synthase (GS/GOGAT) and glutamate dehydrogenase (GDH) pathways. Regulation of these pathways depends on the interaction between different host-fungus combinations (BOTTON and DELL, 1994).

2.2.3.4 Seasonal variation in nutrient uptake

Forest trees take up nutrients mostly from the surface soil where fine roots and mycorrhizae are most abundant, and nutrients and organic matter are concentrated. Seasonal fluctuations in moisture and temperature of the surface soil exert a strong effect on nutrient uptake by eucalypt roots and will be most pronounced in areas with strong seasonal variation (GROVE and MALAJCZUK, 1992). The periodicity of fine root growth and the restricted period of nitrogenase activity in the nodules of eucalypt forest legumes suggest that much of the annual uptake of nutrients is in spring and early summer (DELL and WALLACE, 1983).

Significant seasonal variation in concentrations of nutrients in eucalypt foliage has been observed, although the trends have not been examined in relation to nutrient uptake, root activity or variation in soil moisture and temperature, as has been done with other evergreen tree genera (SCHONAU, 1981, BELL and WARD, 1984). Increased concentrations of nitrogen in foliage of six-year-old *E. saligna* Sm. and *E. wandoo* Blakely were observed in spring, corresponding with the period when fine root development and microbial action were high (DELL and WALLACE, 1983).

2.2.3.5 Nutrient distribution and storage

The distribution of nutrients within the eucalypts can be indicative of the importance of different tissues in the storage of nutrients and of the efficiencies in nutrient use. There is little information on the distribution and storage of inorganic and organic forms of nutrients in eucalypts, particularly nitrogen (GROVE *et al.,* 1996).

MULLIGAN (1988) quantified the concentrations and distribution of the major inorganic and organic forms of phosphorus in relation to the phosphorus supply in seedlings of three eucalypt species, but most studies have only focused on total concentrations of nutrients.

Differences between nutrients in their distribution are related to differences in their physiological function and in their relative mobility within the plant. The distribution of nutrients between components of the tree also varies markedly between species, with the age or size and as a result of changes in the external nutrient supply.

2.2.3.6 The effect of tree species

GROVE *et al.* (1996) indicate that the nutrient content of older trees, and the distribution of nutrients between tree components, varies between eucalypt species, but there are greater differences between eucalypts and other tree genera of comparable biomass. Although the range in nitrogen content of eucalypts is similar to those of other tree genera, the greater biomass of eucalypts indicates that they have a lower average nitrogen concentration.

LAMBERT (1981a) reported that phosphorus levels vary markedly between tree genera, with eucalypts having a much lower content than other tree genera. The calcium content is more variable than other nutrients among tree species. The eucalypts with higher calcium contents are those which have decorticating bark and where a high proportion of the total calcium content of the tree is contained in the bark.

Foliage represents a small proportion of the biomass of older trees but contains a major proportion of nutrients. The average biomass of foliage is approximately 2% of the total biomass of eucalypts but contains 20% of the nitrogen and 17% of the phosphorus in above ground components. By contrast, in four coniferous species, foliar biomass is on average 7% of the total biomass and contains 40% of the nitrogen and 45% of the phosphorus in the above ground biomass. This large percentage of nutrients in coniferous foliage, and the greater longevity of foliage in conifers, as compared to eucalypts, indicates its significance as a sink for nutrients and a major source of nutrients for new shoot growth. Data suggests that the wood and bark of the bole and branches are a commensurately more important store of nutrients in eucalypts than in conifers, and may have a more significant role in biochemical cycling of nutrients (FIFE and NAMBIAR, 1984, NAMBIAR and FIFE, 1991).

GROVE *et al.* (1996) state that gradients in nutrient concentrations within the tree canopy, and differences in trends among nutrients, are explained by an increase in **sclerophylly** (lignin and cellulose contents) with leaf age, and by differences between nutrients in their function and mobility within the tree. SPECHT and RUNDLE (1990) observed that concentrations of phosphorus and other nutrients in a range of Australian plants were negatively correlated to

the lignin and cellulose content of the leaves. GROVE (1990) reported that decreases in concentrations of nitrogen and phosphorus in mid-canopy leaves of *E. diversicolor* over a three year period were associated with a 67% increase in specific leaf mass (g/m^2). In addition to the effects of changes in leaf thickness and the amounts of structural constituents on gradients in foliar concentrations of nitrogen and phosphorus in eucalypts, retranslocation from old leaves to supply the requirements of new shoots is also likely to contribute to trends. In contrast, calcium which shows the reverse trend to nitrogen and phosphorus is relatively immobile within the plant and continues to accumulate in leaves as they age. Variation in calcium concentrations therefore reflects more closely the variation in age. This correlation is also directly applicable to the *E. grandis x E. nitens* hybrid and the same results have been found through foliar analysis data from trial results.

Data from studies indicate that the root systems of eucalypts are more extensive and contain a much greater proportion of total nutrients in environments where either nutrients or water supply are most limiting to growth. This greater allocation of assimilates to roots and the increased proportion of nutrients retained in the roots is a common response of plants to low nutrient supply and has been observed over a range of species growing under stress (CLARKSON and HANSON, 1980).

In seedlings of a fast growing eucalypt, *E. pilularis* Sm., phosphorus deficiency increased the retention of phosphorus in roots and allocation of carbon to roots at an early stage in development. Subsequent increases in the uptake of phosphorus have been attributed to the formation of ectomycorrhizae and resulted in increased transfer of phosphorus to the shoots and an increase in leaf growth (MULLIGAN and PATRICK, 1985).

2.2.3.7 Nutrient supply

Variation in the supply of nutrients due to differences in soil nutrient status affects the distribution of nutrient between tree components. Increased nutrient supply changes the distribution by preferential accumulation of those nutrients in specific sinks or indirectly through effects on growth and the partitioning of dry matter. Increases in the external supply of nitrogen or phosphorus to young seedlings of *E. grandis* decreases the dry mass of roots relative to leaves, although partitioning of dry matter between leaves and stems is not affected (CROMER and JARVIS, 1990).

Growth responses of eucalypt seedlings to the application of phosphorus are generally associated with increased phosphorus concentrations throughout the plant. However, increases in phosphorus concentrations are much larger in stem tissues, particularly the bark, than in leaves; with an increasing supply of phosphorus, a higher proportion of the phosphorus content of shoots is contained in stems (GROVE *et al.,* 1996). In recoppiced *E. diversicolor* forests in South West Australia, the application of phosphorus fertiliser increased the concentrations of phosphorus in all above ground components of the trees, but were proportionally higher in twigs, bark and stem wood than in leaves. The application of nitrogen also increased concentrations of nitrogen in each of these plant tissues but the increases in woody tissues were less marked than for phosphorus (GROVE,1990).

2.2.3.8 Nutrient retranslocation from leaves, wood and bark

FIFE and NAMBIAR (1984) state that most information on the retranslocation of nutrients in eucalypts has been obtained from measurements of withdrawal of nutrients from the leaves during senescence and studies of other forest species suggest that there may also be significant retranslocation of nutrients from younger foliage and fine roots. SPECHT and GROVES (1966) report that the proportion of nitrogen and phosphorus remobilized from senescing leaves is greater in eucalypts than in annual crop species (Table 2.4).

Table 2.4 The remobilisation of nutrients from leaves of eucalypts and other selected forest tree species in the period before litter fall. Nutrient withdrawal calculated as percentage change in concentration between green leaves (G) and freshly senescent leaves (S). Nutrient withdrawal data in grey background

		Nutrient Concentration (%) and Nutrient Withdrawal					
Species	**Leaf stage**	**N**	**P**	**K**	**S**	**Ca**	**Mg**
E. diversicolor	G	1.170	0.058	0.920	0.105	0.820	0.280
	S	0.610	0.021	0.250	0.085	1.090	0.280
		48	64	73	19	-33	0
E. marginata	G	0.840	0.041	0.570	0.103	0.580	0.430
	S	0.300	0.009	0.260	0.085	0.740	0.400
		64	78	54	28	-28	7
E. regnans	G	1.520	0.117	1.130	-	0.230	0.250
	S	0.330	0.035	0.290	-	0.560	0.200
		78	70	74	-	-144	20
Pinus radiata	G	1.180	0.089	0.660	-	0.400	0.180
	S	0.710	0.037	0.110	-	0.960	0.200
		40	58	83	-	-140	-11
Pinus elliottii	G	0.610	0.043	0.160	-	0.610	0.150
	S	0.300	0.009	0.030	-	0.740	0.160
		51	79	81	-	-21	-7

(Modified from GROVE *et al.,* 1996)

Changes in concentration of nutrients from mature, green leaves to freshly senescent leaves provide only an approximation of the amount of nutrients remobilised from foliage and contributing to tree growth through internal nutrient cycling. These estimates do not adequately account for increased sclerophylly and consequent dilution of nutrients, with the ageing of leaves, or for variation in nutrient content with ontogeny and with seasonal fluctuations in the uptake of nutrients (MAGGS,1985).

Studies on the withdrawal of nutrients from eucalypt leaves have not quantified changes to other leaf characteristics (e.g. thickness, content of structural constituents) or to nutrient content per unit of leaf. Concentrations of mobile nutrients within leaves at any one time depend primarily on the net effect of inflow of xylem and efflux through the phloem (HILL, 1980). The variable and generally large gains in the calcium concentrations between mature leaves and senescent leaves of eucalypts (Table 2.4) suggest that age and structural constituents differ widely. These effects can lead to errors in estimates of remobilisation of mobile elements. The proportion of a nutrient remobilised during leaf senescence depends on its relative mobility within the phloem and the initial nutrient concentration (LONERAGAN *et al.*, 1976, CHAPIN and KEDROWSKI, 1983). WOODWELL (1974) states that in hardwoods, the content of mobile nutrients increases rapidly during leaf expansion, reaching a maximum relatively early in the life of the leaf. In contrast, immobile nutrients such as calcium accumulate throughout the life span.

A major component of the biochemical cycling of nutrients in eucalypts is the withdrawal of nutrients from the bark, and from wood during its transition from sapwood to heartwood. An essential difference in the retranslocation of nutrients from leaves is that phloem-immobile elements such as calcium are translocated radially in the stem during heartwood formation and from the outer bark, and are thereby transported to growing tissue, whereas in leaves the calcium remains in senescent tissue. Data suggests that eucalypts are particularly efficient in retranslocating phosphorus from wood during heartwood formation. Nutrient concentrations are greatest near the cambium and, while there are strong gradients from the outer wood to the inner wood, gradients from the inner bark to the outer bark are more variable. Concentrations of nutrients in the heartwood of eucalypts are generally low (GROVE *et al.,* 1996).

Concentrations of other elements also decrease with the transition from sapwood to heartwood in eucalypts. Gradients for potassium are often as large as those for phosphorus, whilst limited data indicates a smaller difference for nitrogen (BEADLE and WHITE, 1968, HINGSTON *et al.*,

1979, LAMBERT, 1981b). The concentration of calcium decreases from sapwood to heartwood in many but not in all eucalypts (Table 2.5).

Table 2.5 Concentration of major nutrients in sapwood, heartwood and bark of eucalypts and other forest species. For some species, concentrations in bark or sapwood are the means of concentrations in inner and outer samples. Heartwood (H), Sapwood (S), Bark (B)

	Concentration (%)											
Species		N			P			K			Ca	
	H	S	B	H	S	B	H	S	B	H	S	B
E. saligna	0.120	0.200	0.300	<0.001	0.008	0.020	0.006	0.075	0.440	0.025	0.064	3.880
E. maculata	0.100	0.180	0.270	<0.001	0.005	0.011	0.022	0.080	0.190	0.237	0.124	2.930
E. diversicolor	0.070	0.120	0.130	0.001	0.008	0.007	0.018	0.105	0.145	0.018	0.064	0.310
E. grandis	0.150	0.310	0.240	<0.001	0.013	0.012	0.020	0.125	0.175	0.075	0.065	3.150
E. obliqua	-	-	-	0.002	0.013	0.014	0.006	0.059	0.266	0.008	0.022	0.430
E. marginata	0.050	0.100	0.210	<0.001	0.005	0.012	0.023	0.051	0.234	0.007	0.024	0.430
E. viminalis	0.090	0.200	0.270	0.014	0.016	0.020	0.007	0.115	0.160	0.020	0.073	2.240
Pinus radiata	-	-	-	0.0007	0.0050	0.0390	0.0280	0.0940	0.0730	0.1150	0.0900	0.5600

(Modified from GROVE *et al.*, 1996)

LAMBERT (1981b) wrote that the extent of retranslocation of calcium from heartwood may vary markedly for species on different sites. With *E. obliqua*, concentrations of calcium decreased by ten times from sapwood to heartwood on some sites but did not vary through the stem wood at others. MARSCHNER (1999) states that the mechanisms for the retention and remobilisation of calcium within the stem are not well understood; however, exchange adsorption is important in long distance calcium transport and explains why movement is not directly related to rates of water transport.

The increased uptake of nutrients as a result of applying fertilisers can markedly increase the nitrogen and phosphorus contents of the stem, but the changes in eucalypts are greatest close to the cambium. It is likely that much of the increased phosphorus storage is in a form that is readily mobilised (MULLIGAN, 1988).

Where uptake of nitrogen and phosphorus exceeds current requirements for growth for only a short period, due either to fluctuating seasonal conditions or to the short term effectiveness of applied fertiliser, this store of nutrients in the stem may be important as a source of nutrients to sustain growth (GROVE *et al.*, 1996).

Retranslocation of nutrients from outer bark may also be a significant component of biochemical cycling in some eucalypts. Average concentrations of nutrients are often much

greater in bark than in stem wood and although bark is less than 30% of the total stem biomass, it contains a larger proportion of the nutrient content of the whole stems (BEADLE and WHITE, 1968, LAMBERT, 1981b).

2.2.3.9 The biochemical cycle and annual nutrient requirements

In mature *E. obliqua* and a 27-year-old forest of *E. grandis*, between 45% and 55% of the total gross annual requirement for phosphorus and one third of the annual requirement for nitrogen is met by biochemical cycling (TURNER and LAMBERT, 1983, BAKER and ATTIWILL, 1985). In *E. obliqua,* approximately 25% of the potassium and magnesium requirements, but only 2% of the calcium requirements, are met by internal redistribution (ATTIWILL, 1980). This small redistribution of calcium shows that *E. obliqua* depends on a continuous uptake of calcium from the soil to meet the requirements of new shoot growth. There was also no net redistribution of calcium in a fast growing *E. grandis* stand, although remobilisation from the bark and heartwood amounted to 22% of the gross annual calcium needs (TURNER and LAMBERT, 1983).

GROVE *et al.* (1996) suggest that for eucalypts, where there are marked differences in calcium concentrations between heartwood and sapwood and between inner and outer bark, remobilisation of calcium within stems and branch tissues is likely to be a major source of calcium to meet the requirements of new shoot growth, particularly where the uptake of calcium by fine roots is limited by seasonal drought.

Retranslocation of nutrients from leaves forms the major component (45-92%) of the biochemical cycling of nitrogen, phosphorus and potassium in eucalypts (ATTIWILL, 1980, TURNER and LAMBERT, 1983). Redistribution of these nutrients during the transition to heartwood is also a significant component of the biochemical cycle and redistribution from outer bark and twigs make small contributions. Retranslocation of nutrients from stems is less significant in biochemical cycling than the withdrawal of nutrients from leaves, although the extent of nutrient storage and retranslocation in bark, branches and twigs is difficult to quantify and to date may have been under estimated (GROVE *et al.*, 1996). ATTIWILL (1980) summarised data from a range of forest ecosystems showing that hardwoods, including eucalypts, tend to retain a greater proportion of their annual uptake of phosphorus than conifers (Table 2.6). This high retention of phosphorus, and the generally low phosphorus content of eucalypts together indicate effective mechanisms for conserving and utilising absorbed phosphorus.

Table 2.6 Retention of nutrients by *E. obliqua* as compared to other forest species

Retention is the annual accumulation of nutrients in the stand; uptake is retention plus the amount of nutrient in the biogeochemical cycle

	Ratio of retention : uptake		
	P	K	Ca
Pines	0.19	0.19	0.16
Other conifers	0.18	0.30	0.16
Hardwoods	0.42	0.35	0.40
E. obliqua	0.34	0.23	0.17

(ATTIWILL, 1980)

The combined effect of a number of mechanisms which enhance the acquisition and conservation of nutrient elements enables eucalypts to endure low levels of nutrient supply in the soil. The efficiency of these mechanisms in the uptake and utilisation of nutrients is undoubtedly a major reason for the success of eucalypts as a plantation species world wide when planted on nutrient-poor and degraded sites. Furthermore many eucalypts have the potential for increased growth and storage of nutrients when fertilised with the major nutrients, nitrogen and phosphorus, indicating the considerable plasticity of the eucalypts in adjusting to changes in supply (GROVE *et al.*, 1996).

2.2.4 The nutritional physiology of the eucalypts - nutrition and growth

2.2.4.1 Canopy response to nitrogen and phosphorus nutrition

The development of shoots in eucalypts is opportunistic and indefinite. During a growth flush, the number of leaves on a shoot can increase exponentially by virtue of a distinctive phyllotaxy:

1) Terminal and axillary meristems both retain unlimited capacity to form leaf primordia.
2) As each leaf unfolds, a naked axillary bud is formed with the capacity for further rapid and indefinite development of new shoots.
3) Accessory tissue at the base of each axillary bud retains a latent potential for meristematic activity and organogenesis if other growing points fail. For example, proliferation of epicormic shoots following severe drought, defoliation by insects or fire, can be traced to earlier generation of such accessory tissue (KRIEDEMANN and CROMER, 1996).

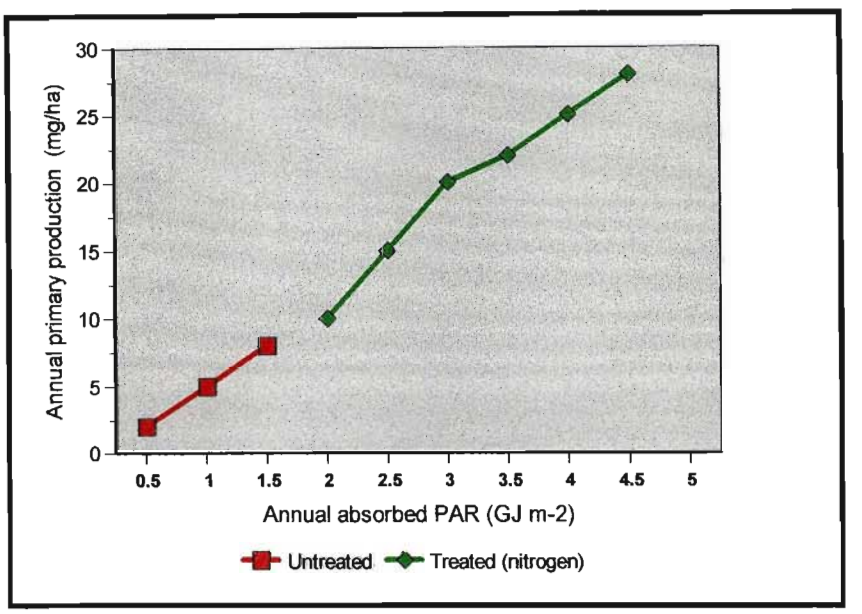
Where growing conditions are conducive to apical dominance, axillary buds and accessory tissue remain quiescent and growth is monopodial. Additional nutrients can stimulate axillary growth with branching to the second and third orders.

Canopy assimilation by *E. grandis* is a direct function of absorbed photosynthetic active radiation (PAR) but the slope of that relationship is nitrogen dependent (Figure 2.3). In the

'top-down' modelling approach of LANDSBERG and McMURTRIE (1985), photosynthetic response to leaf nitrogen must be taken into account because variation in that term will affect **e** in the expression: $Dw_i = \mathbf{e}\ Q\ Dt_i$

Where Dw_i is dry mass increment over a time interval Dt_i, Q is absorbed PAR and **e** is efficiency of utilisation of radiation. Appropriate **e** values for treated versus control can be inferred from the slope of relationships between annual primary production and annual Q.

Figure 2.3 Estimated net above ground primary production of *E. grandis* as a function of mean annual absorbed PAR during the first three years



(Modified from LEUNING *et al.*, 1991)

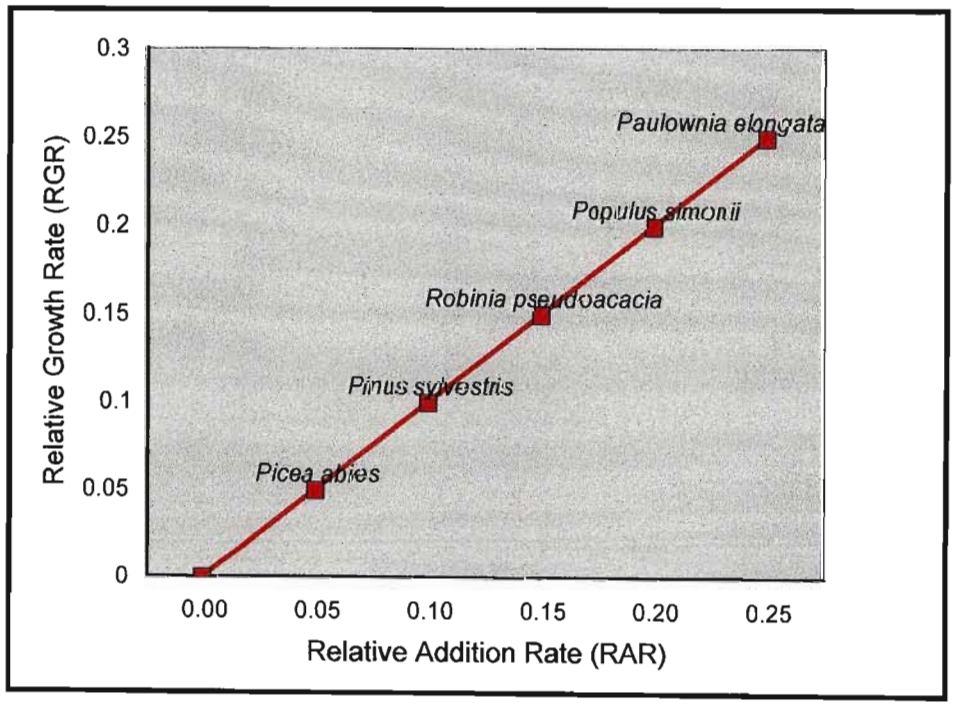
Taking regression slopes for annual primary production in Figure 2.3 as indicative of energy conversion, **e** was 0.44 MJ for low nutrient trees compared with 0.76 MJ for high nutrient trees (LEUNING *et al.*, 1991).

The present limited data suggests that nutrient-use efficiency for wood production is a consequence of two sets of interacting processes, **assimilation and partitioning**, which are both nutrient sensitive. Positive effects of increased nutrient supply on assimilation by the leaves, and especially on leaf area, are compounded by an increase in stem growth with relatively less root growth and turnover (KRIEDEMANN and CROMER, 1996).

2.2.4.2 Seedling growth - nutrient culture and growth analysis

Tree seedlings will grow exponentially for some weeks when space and nutrients are carefully managed. An ordered ontogeny can be driven at a predetermined relative growth rate (RGR) by a judicious supply of nutrients. In effect, steady-state exponential growth is set by the relative addition rate (RAR) of a key nutrient, while other essential nutrients are kept non-limiting. In this method, seedlings are commonly held in aeroponic spray chambers where a small volume of liquid is continuously circulated and further nutrients are supplied at predetermined relative addition rates (INGESTAD and LUND, 1986). Relative addition rate (RAR) thus drives RGR which increases linearly with RAR up to the point of nutrient saturation. Maximum RGR also depends upon PAR and CO_2, but genetic factors eventually limit growth (Figure 2.4).

Figure 2.4 Linear relationship between the maximum relative growth rate achieved by seedlings of various species and the corresponding relative rate at which nutrients were supplied



(KRIEDEMANN and CROMER, 1996).

Relative growth rate (RGR) for *E. grandis* seedlings under natural daylight in summer can reach 12% per day so that values approaching 25% per day should be possible with continuous light (CROMER and JARVIS, 1990).

2.2.5 Nutrient concentrations in *Eucalyptus* in relation to differences between taxa, sites, and components

NOBLE (1989) reviewed the evidence surrounding the hypothesis that the subgenera *Monocalyptus* and *Symphyomyrtus* are physiologically and ecologically distinct. He concluded that, as a general rule, *Symphyomyrtus* species grew on more fertile soils than *Monocalyptus* species and that the growth responses of *Symphyomyrtus* species to added nutrients are greater than those of *Monocalyptus* species.

Tables 2.7 summarises the differences between three eucalypt subgenera, the third largest subgenus *Corymbia* (bloodwoods) is also included. All of the data are from native forests since they are not confounded by the effects of nutritional amendment. Both *E. grandis* and *E. nitens* are members of the *Symphyomyrtus* subgenus.

Table 2.7 Mean foliar concentrations of some essential nutrients for *Corymbia, Monocalyptus* and *Symphyomyrtus* species. Mean values ± standard errors. Values with different letter suffixes are significantly different, $P < 0.01$

Nutrient	Subgenus		
	Corymbia	*Monocalyptus*	*Symphyomyrtus*
N (%)	1.18 ± 0.07	1.19 ± 0.04	1.21 ± 0.04
P (%)	0.05 ± 0.005a	0.072 ± 0.003b	0.069 ± 0.03b
K (%)	0.60 ± 0.09ab	0.46 ± 0.02a	0.71 ± 0.03b
Ca (%)	0.48 ± 0.07a	0.48 ± 0.03a	1.00 ± 0.07b
Mg (%)	0.26 ± 0.02	0.30 ± 0.02	0.26 ± 0.01
Mn (mg/kg)	190 ± 50a	340 ± 50a	610 ± 70b

(JUDD *et al.*, 1996)

Note: Table 2.7 is included as baseline data for the study of foliar concentrations of *Symphyomyrtus* (*E. grandis* and *E. nitens*) as there is very little information on the nutrient concentrations of either eucalypt clonal field hedges or hydroponic mini-hedges. Readers should be aware that Table 2.7 is from a study of mature trees and the physiology may differ to those of clonal hedge plants maintained on a continual cycle of nutrient feed.

LAMBERT (1981) states that mean concentrations of calcium and manganese in foliage are significantly greater in *Symphyomyrtus* than in *Monocalyptus* and *Corymbia* (Table 2.7). JUDD *et al.* (1996) indicate that of the other macro-nutrients in foliage, the concentration of phosphorus does not differ significantly between *Symphyomyrtus* and *Monocalyptus*, but is lower in *Corymbia*. The mean concentration of potassium in foliage is significantly greater in

Symphyomyrtus than in *Monocalyptus*, but there are no significant differences in nitrogen and magnesium between the three subgenera (Table 2.7).

It has been reported that nutrient concentrations of calcium in the bark of smooth barked species are greater than in fibrous barked species (WISE and PITMAN, 1981). The mean concentration of calcium in the bark of smooth barked species is five times greater than that of rough barked species. Concentrations of phosphorus, potassium and magnesium in the bark of smooth barked species are 1.5 to 3 times those of fibrous barked species (Table 2.8).

Table 2.8 Foliar concentrations of macro-nutrients in bark of fibrous barked and smooth barked eucalypts. Mean values ± standard errors. All comparisons are significantly different, $P<0.001$

	Bark type	
Nutrient	Fibrous	Smooth
N (%)	0.24 ± 0.02	0.21 ± 0.01
P (%)	0.014 ± 0.01	0.022 ± 0.01
K (%)	0.17 ± 0.01	0.32 ± 0.02
Ca (%)	0.42 ± 0.05	2.21 ± 0.13
Mg (%)	0.07 ± 0.01	0.19 ± 0.01

(LAMBERT, 1981b)

LAMBERT and TURNER (1983) suggest that if the differences in calcium and manganese concentrations, and to a lesser extent, in potassium and magnesium concentrations, between subgenera are general responses, one could expect substantial differences in nutrient concentrations when comparing individual species on a given site. It has been shown that the foliar concentrations of potassium and calcium were significantly greater for *E. nitens* (Deane & Maiden) Maiden (*Symphyomyrtus*) and less for magnesium and nitrogen than for *E. regnans* (*Monocalyptus*) (Table 2.9). Nutritional differences are not restricted to subgenera, and there may be significant differences within subgenera and even between closely related species.

Table 2.9 Concentrations of macro-nutrients in foliage at age two-years for adjacent *E. regnans* and *E. nitens* plantations grown on an unamended site with uniform site preparation in south Gippsland, Victoria. * Significant differences, $P<0.005$

Nutrient	*E. regnans*	*E. nitens*
N (%)	2.25*	1.83
P (%)	0.11	0.11
K (%)	0.52	0.88*
Ca (%)	0.15	0.44*
Mg (%)	0.19*	0.14

(JUDD *et al.*, 1996).

2.2.6 Site differences

2.2.6.1 Natural forest versus plantation

From a broad base of nutrient comparisons between natural forests and plantations, it is apparent that both maximum and typical concentrations of all elements (except manganese) in foliage are greater in plantations than in native forests (Table 2.10).

Table 2.10 Ranges of foliar concentrations for native forest and plantation eucalypts

	Native forest		Plantation	
Element	Range	Typical range	Range	Typical range
N (%)	0.70 - 2.10	0.80 - 1.50	0.11 - 3.36	1.00 - 2.30
P (%)	0.006 - 0.180	0.050 - 0.100	0.011 - 0.980	0.050 - 0.150
K (%)	0.06 - 1.29	0.50 - 0.80	0.10 - 3.03	0.40 - 1.40
Ca (%)	0.03 - 1.71	0.40 - 0.80	0.11 -5.41	0.50 - 1.00
Mg (%)	0.04 - 0.62	0.20 - 0.35	0.08 - 1.73	0.20 - 0.40
Fe (mg/kg)	10 - 330	50 - 100	20 - 3500	50 - 250
Mn (mg/kg)	30 - 1330	200- 700	10 - 4050	100- 1000
Na (mg/kg)	100 - 4100	1500 - 3000	200 - 13600	500 - 5000
S (mg/kg)	100 -1400	800 -1000	600 -2800	1000 - 2000
Cl (mg/kg)	1100 - 10500	3000 - 5000	2800 - 18100	4000 - 10000
Al (mg/kg)	10 - 600	100 -150	7 - 274	40 - 200
Cu (mg/kg)	1 - 11	2 - 7	2 - 18	4 - 10
Zn (mg/kg)	5 - 52	10 - 25	6 - 59	15 - 40
B (mg/kg)	62 - 157	75 - 125	15 - 84	20 - 50

(JUDD *et al.*, 1996)

JUDD *et al.* (1996) state that the greater nutrient concentrations reflect increased nutrient availability in plantations due to intensive site preparation, additions of fertiliser and other silvicultural treatments (Tables 2.10 and 2.11). The high concentrations of foliar nitrogen (>2.0%), phosphorus (>0.1%) and potassium (>1.0%), which are typical of many plantations, probably indicate luxury consumption and result from the common practice of applying fertiliser, typically as some form of N.P.K formulation, during establishment of the plantation. Conversely, the substantially lower minimum and typical ranges for boron in plantations than in native forests suggest that boron may be deficient or sub-optimum in many plantation soils. Given the extent of the variation, it must be concluded that site fertility is more important in determining nutrient concentrations than are taxonomic relationships (JUDD *et al.*, 1996).

Table 2.11 Mean concentrations of nutrients in *E. grandis* foliage from young trees sampled in plantations and from seedlings - glass house experiments

	Native forest			Plantation	
Element	Mean ± s.e	CV (%)	n	Range	Typical range
Young trees					
N (%)	2.04 ± 0.08	30	55	0.85 - 3.16	1.25 - 2.75
P (%)	0.133 ± 0.09	50	55	0.011 - 0.270	0.075 - 0.200
K (%)	0.81± 0.04	30	46	0.85 - 3.16	1.25 - 2.75
Ca (%)	0.87 ± 0.05	32	31	0.38 - 1.38	0.50 - 1.10
Mg (%)	0.29 ± 0.02	28	31	0.15 - 0.44	0.22 - 0.35
Fe (mg/kg)	160 ± 20	59	29	60 - 520	100 - 220
Mn (mg/kg)	830 ± 110	67	25	250 - 2170	400 - 1000
Na (mg/kg)	3300 ± 800	71	11	400 - 6300	1500 - 5500
S (mg/kg)	1800 ± 100	29	19	600 - 2600	1300 - 2200
Al (mg/kg)	89 ± 29	74	5	35 - 191	40 - 140
Cu (mg/kg)	10 ± 1	43	25	4 - 18	6 - 14
Zn (mg/kg)	22 ± 1	48	24	13 - 39	15 - 30
B (mg/kg)	24 ± 3	22	3	18 - 27	18 - 27
Young seedlings					
N (%)	2.61 ± 0.18	40	34	0.85 - 5.30	1.25 - 5.30
P (%)	0.200 ± 0.19	55	34	0.018 - 0.520	0.075 - 0.275
K (%)	1.41± 0.11	41	28	0.28 - 2.72	0.75 - 1.75
Ca (%)	0.74 ± 0.04	28	26	0.49 - 1.24	0.55 - 0.95
Mg (%)	0.33 ± 0.01	15	26	0.12 - 0.43	0.25 - 0.40
Fe (mg/kg)	170 ± 7	16	14	120 - 220	150 - 190
Mn (mg/kg)	310 ± 1150	187	16	70 - 1800	80 - 120
Na (mg/kg)	--	--	--	--	--
S (mg/kg)	2600 ± 200	19	7	1700 - 3300	2400 - 2900
Al (mg/kg)	--	--	--	--	--
Cu (mg/kg)	14 ± 0.44	12	14	12 - 16	12 - 16
Zn (mg/kg)	29 ± 1.37	18	14	16 - 38	25 - 32
B (mg/kg)	120 ± 30	93	14	10 - 374	55 - 239

(JUDD *et al.*, 1996)

2.2.7 Eucalypts response to different continental sites

JUDD *et al.* (1996) record that although a range of eucalypt species may be grown in any given country, most plantings and nutritional studies, tend to be of only one or two species which may not be grown extensively anywhere else. Sufficient data does exist to allow comparisons between a few species in Australia and elsewhere. Although there is a large variation in concentrations, the ranges of individual elements for each species tend to overlap, suggesting that eucalypts behave much the same nutritionally whether planted in Australia or abroad (Table 2.12).

Table 2.12 Analyses of foliage samples of *E. grandis and E. saligna* by different laboratories around the world

Country	N	P	K	Ca	Mg
E. grandis					
Australia	1.09 - 2.56	0.09 - 0.21	0.65 - 0.75	0.45 - 0.48	0.23 - 0.27
Brazil	1.84 - 2.26	0.11 - 0.17	0.38 - 1.14	0.38 - 1.15	0.15 - 0.44
South Africa	0.85 - 3.12	0.01 - 0.26	0.36 - 1.35	0.48 - 1.22	0.22 - 0.40
E. saligna					
Australia	0.80 - 2.15	0.03- 0.18	0.70 - 1.80	0.31 - 1.60	0.22 - 0.40
USA (Hawaii)	0.69 - 2.94	0.05 - 0.20	0.40 - 1.42	0.56 - 1.06	0.19 - 0.36
Brazil	0.29 - 1.91	0.05 - 0.12	0.22 - 0.73	0.23 - 0.70	0.05 - 0.36

(Modified from JUDD *et al.*, 1996)

2.3 Concepts of mineral nutrition

2.3.1 The role of nutrients

The composition of fresh plant matter includes approximately 80 to 95% water. The exact percentage depends on the plant species and the turgidity of the plant at the time of sampling. Due to this variability, chemical analyses are based on the more stable dry matter. Over 90% of the dry mass of plant matter consists of the three elements: carbon (C), oxygen (O) and hydrogen (H). If only 15% of the fresh mass of a plant is dry matter and 90% is represented by carbon, oxygen and hydrogen, then all the remaining elements account for approximately 1.5% of the fresh mass of the plant (0.15 x 0.10) (RESH,1998).

According to MARSCHNER (1999), a mineral nutrient can function as a constituent of an organic structure, as an activator of enzyme reactions, or as a charge carrier and osmoregulator. The main functions of mineral nutrients such as nitrogen (N), sulphur (S), and phosphorus (P) are to act as constituents of proteins and nucleic acids. Mineral nutrients such as magnesium (Mg) and the micro-nutrients (excluding chlorine) function as constituents of organic structures, predominantly the enzyme molecules, where they are directly or indirectly involved in the catalytic function of the enzymes. Potassium (K) and chlorine (Cl) are the only mineral nutrients that are not constituents of organic structures. They function mainly in osmoregulation, the maintenance of electrochemical equilibrium in cells and the regulation of enzyme activities. Due to their low concentrations, micro-nutrients do not play a direct role in either the osmoregulation or the maintenance of electrochemical equilibrium.

Only 16 elements are generally considered essential for growth of higher plants (Table 2.13). They are divided into macro-nutrients required in large quantities and micro-nutrients needed in considerably smaller quantities.

Table 2.13 Elements essential for most higher plants

Element	Symbol	Available form	Atomic mass	ppm	Concentration in dry tissue %
Hydrogen	H	H_20	1.1	60,000	6.00000
Carbon	C	CO_2	12.01	450,000	45.00000
Oxygen	O	O_2, H_2O	16	450,000	45.00000
		Macronutrients			
Nitrogen	N	NO_3^-, NH_4^+	14.01	15,000	1.50000
Phosphorus	P	$H_2PO_4^-$, HPO_4^{2-}	30.98	2,000	0.20000
Potassium	K	K^+	39.1	10,000	1.00000
Magnesium	Mg	Mg^{2+}	24.32	2,000	0.20000
Calcium	Ca	Ca^{2+}	40.08	5,000	0.50000
Sulphur	S	SO_4^{2-}	32.07	1,000	0.10000
		Micronutrients			-
Iron	Fe	Fe^{3+}, Fe^{2+}	55.85	100	0.01000
Boron	B	BO_3^{3-}, $B_4O_7^{2-}$	10.82	20	0.00200
Chlorine	Cl	Cl^-	35.46	100	0.01000
Manganese	Mn	Mn^{2+}	54.94	50	0.00500
Zinc	Zn	Zn^{2+}	65.38	20	0.00200
Copper	Cu	CU^{2+}, CU^+	63.54	6	0.00060
Molybdenum	Mo	MoO_4^{2-}	95.96	0	0.00001

(RESH, 1998)

The concentration required in plants for normal growth and metabolism varies enormously among the essential elements (Table 2.13). Many of the essential elements were not identified until the purification of reagent chemicals was developed and the techniques of analytical chemistry had brought the detection limits below the milligram level (BENTON-JONES, 1998). The role of these essential elements is summarised in Table 2.14 below:

Table 2.14 Essential elements, their forms for uptake and functions in the plant

Essential element	Form of uptake	Functions in plant metabolism
C, H, O, N, S	Ions in solution (HCO_3^-, NO_3^-, NH_4^+, SO_4^{2-}) or gases in the atmosphere (O_2, N_2, SO_2)	Major constituents of organic substances
P, B	Ions in solution (PO_4^{3-}, BO_3^{3-})	Energy transfer reactions and carbohydrate movement
K, Mg, Ca, Cl	Ions in solution (K^+, Mg^{2+}, Ca^{2+}, Cl^-)	Non-specific functions, or specific components of organic compounds, or maintain ionic balance
Cu, Fe, Mn, Mo, Zn	Ions or chelates in solution (Cu^{2+}, Fe^{2+}, Mn^{2+}, MoO^-, Zn^{2+})	Enable electron transport and catalysts for enzymes

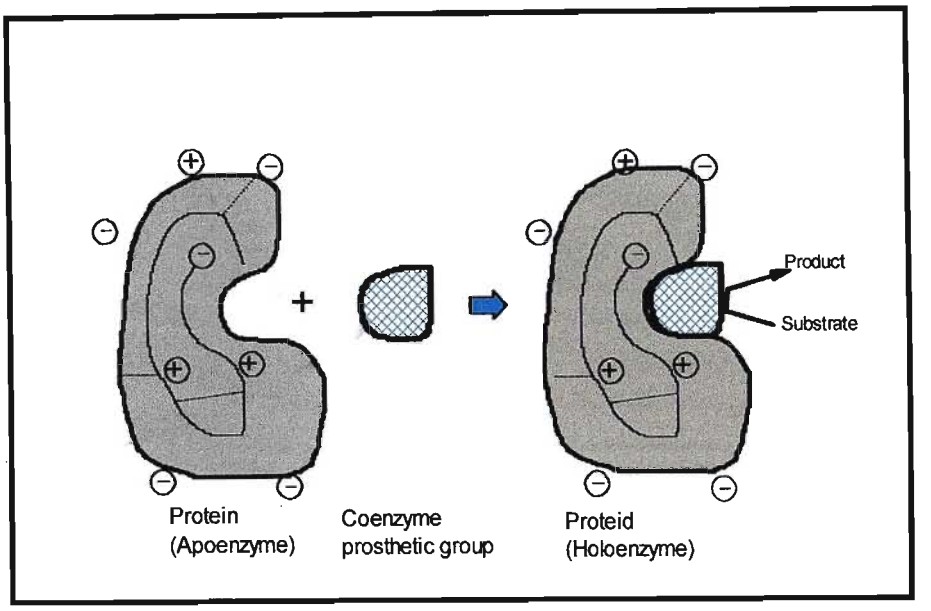
(BENTON-JONES, 1998)

2.3.1.1 The role of macro-nutrients

Nitrogen and sulphur are integral constituents of protein structures and thus of *apoenzymes.* For the catalytic reaction of the majority of enzymes, a non-protein (a coenzyme) and a prosthetic group (metal component) is required (Figure 2.5). Typical coenzymes include ATP

and FAD whilst the prosthetic groups include chlorophyll, cytochromes and nitrogenase in which a metal acts as a functional group.

Figure 2.5 Components of an enzyme molecule. The shaded area represents the hydration shell of water molecules



(MARSCHNER, 1999)

Most of the metal atoms integrated into metalloproteins are transition metals, which perform their catalytic function through a change in valency. Examples include iron in cytochromes, copper in plastocyanin, and molybdenum in nitrogenase. In some enzymes though, the metal performs its catalytic function by forming an *enzyme-substrate-metal* complex such as magnesium in ATPase (MARSCHNER, 1999).

2.3.1.2 Role of micro-elements in plants

Many physiologists believe that more than the 16 essential elements must be present to ensure the normal growth and development of a plant (BENTON-JONES, 1998). Various terms have been used to identify them, some as **heavy metals** (their molecular weight >50) or by the more general term **trace elements** or micro-elements. In most instances, very little is known of their effect on plant growth and metabolic functions. In earlier hydroponic culture of plants, an **'A-Z'** solution containing 20 additional elements was added to the nutrient solution already containing the known **essential** elements (Table 2.15). The idea was to ensure that almost every element found in soil was incorporated into the solution.

Table 2.15 Elements included in the 'A-Z' solution

Element (symbol)	Element (symbol)
Aluminium (Al)	Lithium (Li)
Arsenic (As)	Lead (Pb)
Barium (Ba)	Mercury (Hg)
Bismuth (Bi)	Nickel (Ni)
Bromine (Br)	Rubidium (Rb)
Cadmium (Cd)	Selenium (Se)
Chromium (Cr)	Strontium (Sr)
Cobalt (Co)	Tin (Sn)
Fluorine (F)	Titanium (Ti)
Iodine (I)	Vanadium (V)

(BENTON-JONES, 1998)

There is a considerable difference in how elements are identified as **essential** for animals as compared to plants (BENTON-JONES, 1998). Elements that enhance animal growth and function, but will not result in death if absent, are classified as **essential.** If the criterion of **growth enhancement** is applied to plants, another class of elements can be established, as there are elements that will enhance plant growth when present as compared to plant performance when absent. There are two kinds of response due to the presence of those elements that have a beneficial effect :

- A direct effect related specifically to that element, and
- Enhancement of growth by means of substitution for an essential element.

An element that describes the first effect is silicon (Si), which enhances the growth and appearance of rice. In its absence, rice plants lodge readily and lack stem sturdiness. Two other effects of Si are disease resistance - the presence of Si is frequently associated with fungus resistance - and enhancement of aluminium and iron tolerance by plants. EPSTEIN (1994) suggests that Si is **essential** for some plants. Experimentation has also concluded that titanium (Ti) can enhance plant growth when applied in a chelated form.

An example of the second effect for a beneficial element is sodium (Na), which can enhance plant growth and vigour of some crops, as sodium acts as a partial substitute for potassium (K). A similar substitution has been suggested for molybdenum (Mo) with vanadium (V) (BENTON-JONES, 1998).

Table 2.16 lists the elements and their average concentrations whilst Table 2.17 is a summary of the functions of the essential elements in higher plants.

Table 2.16 Trace element content in mature leaf tissue for a variety of plant species

Element (symbol)	Mean: mg/kg dry mass.	Sufficient to toxic range: mg/kg	Excessive mg/kg	Element (symbol)	Mean: mg/kg dry mass.	Sufficient to toxic range: mg/kg	Excessive mg/kg
Arsenic (As)	0.10	1-1.7	15-20	Iodine (I)	3.00		
Antimony (Sb)	0.10	7-50	150.00	Lead (Pb)	1.00	5-10	30-300
Barium (Ba)	40.00		500.00	Mercury (Hg)	0.10	-	1-3
Beryllium (Be)	0.00			Nickel (Ni)	1.50	0.5-5	10-100
Bismuth (Bi)	0.01			Selenium (Se)	0.02	0.001-2	5-30
Bromine (Br)	4.00			Silver (Ag)	0.20		
Cadmium (Cd)	0.05	0.05-0.20	5-30	Strontium (Sr)	50.00		
Cerium (Ce)	0.50			Thallium (Tl)	0.05		20.00
Cesium (Cs)	0.20			Tin (Sn)	0.20		60.00
Chromium (Cr)	1.50	0.1-0.5	5-30	Titanium (Ti)	5.00	0.2-0.5	50-200
Fluorine (F)	2.00	5-30	50-500	Tungsten (W)	0.20		
Gallium (Ga)	0.10			Uranium (U)	0.01		
Gold (Au)	0.00			Vanadium (V)	0.50	0.2-1.5	5-10

(KABATA-PENDIAS and PENDIAS, 1994, MARKERT, 1994)

Table 2.17 Summary of the functions of essential elements in higher plants

Element	Physiological process	Activator of enzyme	Constituent of metabolite or cell component
Nitrogen			Amino acids, proteins, nucleic acids, nucleotides, chlorophyll
Phosphorus	Energy storage and transfer, membrane integrity		ATP, nucleotides, nucleic acids, phospholipids
Potassium	Translocation, water relations, energy relations, stomatal opening, regulation of cellular pH, osmoregulation, cation-anion balance	+	
Sulphur	Protein synthesis and function, energy transfer, structure		Amino acids, co-enzymes, ferredoxins, sulfolipids, proteins
Calcium	Membrane maintenance, cell division and elongation, cell wall stabilisation, cation-anion balance, osmoregulation, second messenger in environmental signals	+	Calcium pectates
Magnesium	CO_2 assimilation, regulation of cellular pH, cation-anion balance, protein synthesis, carbohydrate partitioning	+	Chlorophyll, ribosomes
Chlorine	Maintenance of electrical neutrality, internal turgour		
Copper	Lignin synthesis, terminal oxidation in redox reactions, pollen formation and fertilisation		Ascorbate oxidase, phenol oxidases, cytochrome oxidase, plastocyanin, CuZn superoxide dismutase
Zinc	Auxin metabolism, nucleotide synthesis, membrane integrity	+	Dehydrogenases, CuZn superoxide dismutase, carbonic anhydrase, RNA polymerase, alkaline phosphatatse, phopholipase, carboxypeptidase
Manganese	Oxidation-reduction in electron transport, O_2 evolution in photosynthesis	+	Mn superoxide dismutase
Iron	Oxidation-reduction in electron transport		Iron porphyrins (leaves), ferredoxins
Boron	Nucleotide synthesis, assimilate translocation, cell wall biosynthesis and structure, plasma membrane integrity	+	
Nickel	Urea metabolism via urease	+	
Sodium	Conversion of pyruvate to phosphoenolpyruvate in C_4 photosynthetic pathway		
Molybdenum	Nitrogen fixation, nitrate reduction		Nitrogenase, nitrate reductase, xanthine oxidase/dehydrogenase

(GRUNDON *et al.*, 1997)

2.3.2 Differential nutrient removal from solution

BUGBEE (1996) states that the essential nutrients can be placed into three categories based on how quickly they are removed from solution. Group 1 elements are actively absorbed by roots and can be removed from solution in a few hours. Group 2 elements have intermediate uptake rates and are usually removed from solution slightly faster than water is removed.

Group 3 elements are passively absorbed from solution and often accumulate in solution. Table 2.18 indicates the approximate uptake rates of the essential plant nutrients.

Table 2.18 Nutrient removal categorisation

Group 1	Active uptake, fast removal	NO_3^-, NH_4^+, P, K, Mn
Group 2	Intermediate uptake	Mg, S, Fe, Zn, Cu, Mo, C
Group 3	Passive uptake, slow removal	Ca, B

(BUGBEE, 1996)

One of the problems with individual ion monitoring and control is that the concentration of the group 1 elements (N, P, K, Mn) must be kept low to prevent their toxic accumulation in plant tissue. Low concentrations are difficult to monitor and control. The total amount of nutrients in solution can easily and accurately be determined by measuring the electrical conductivity of the solution. However, because of the differential rate of nutrient uptake, conductivity measurements mostly measure the calcium, magnesium and sulphate remaining in solution. The micro-nutrients contribute less than 0.1% to electrical conductivity (BUGBEE, 1996).

2.3.3 Elemental toxicity

Hydroponic nutrition is extremely susceptible to elemental toxicity as a result of inaccurate elemental concentrations combined during preparation of specific formulations. This is compounded by the absence of a soil substrate that is able to buffer some of the toxic effect through its cation exchange capabilities (BENTON-JONES, 1998).

A number of elements can become toxic to plant material at elevated levels. Most of the micro-nutrients (B, Cl, Cu, Mn and Zn) fall into this category. The toxicity effect may be direct, i.e. the element directly impacts on the plant, or the effect is indirect by reducing the availability of another element or by interfering with a normal physiological process. Boron (B) can significantly reduce the growth of a crop, such as maize, when applied at rates required for a high boron demanding crop such as peanut or cotton. The carry over of copper (Cu) from the long term use of copper based fungicides in orchards and vineyards poses a significant problem for subsequent crops. Manganese (Mn) can be elevated to toxic concentrations in soil when the pH of a mineral soil is very acidic (< pH 5.5). An example of an indirect effect element is zinc (Zn). If present at high concentrations, zinc will interfere with the normal iron (Fe)

metabolism in the plant resulting in typical iron deficiency symptoms. Chlorine (Cl) normally exists in solution as the chloride ion (Cl^-). The chloride effect is due to the presence of salt (NaCl) at concentrations that restrict water and nutrient uptake by roots. The most common non-essential element that is toxic to plants is aluminium (Al), which can reach excessive levels in a soil solution due to soil acidity. It stunts plant growth and interferes with the uptake of phosphorus (P) by root hairs (BENTON-JONES, 1998).

Plants have the ability to adjust to metal-element toxicity through the following mechanisms:

- Selective uptake of ions.
- Decreased permeability of cell membranes.
- Immobilisation of ions in roots, foliage and seeds.
- Removal of ions from metabolism by deposition in fixed and insoluble forms in various organs and organelles.
- Alterations in metabolic patterns - increased enzyme system that is inhibited, or increased antagonistic metabolites, or reduced metabolic pathway bypassing an inhibited site.
- Adaptation to toxic metal replacement of a physiological metal in an enzyme.
- Release of ions from plants by leaching from foliage, guttation, leaf abscission and excretion from root hairs.

Toxicity and tolerance to the metal elements by plants can occur due to elemental interactions, mainly with the major elements calcium and phosphorus. Some researchers suggest that one of the major roles of calcium is to counter the toxicity effect of the heavy metals (BENTON-JONES, 1998).

2.3.4 Nutrient function and behaviour in the plant

SMITH and LONERAGAN (1997) state that the concentrations of most nutrients are restricted to quite a narrow range. Plants have the ability to tune the rate at which they absorb nutrients from their rooting medium to the demand established by their growth rate. Thus, very large changes in concentrations of nutrients around plant roots often result in relatively small changes in tissue concentrations. In their work with dilute flowing culture techniques, ASHER and LONERAGAN (1967) used a 625-fold range of phosphorus concentrations continuously maintained at the root surface. In spite of this, there was only a 10-fold range in phosphorus concentrations of the shoots of plants growing in these solutions.

2.3.5 Nutrient uptake at the cellular level

The uptake of nutrients is mediated by membrane transport proteins embedded in the plasmalemma. The functioning of many of these specific nutrient transporters is linked to proton pumps through cotransporter or antiporter activities. The specificity of these transport proteins, or permeases, and the mechanisms by which they are regulated, control the entry of inorganic ions into the symplasm of the roots. Many of the 'high affinity' permeases associated with the uptake of major nutrients in field grown plants are subject to feedback regulation. Thus, the ion transport function is repressed in plants supplied with normal nutrient supply and de-repressed when the nutirent supply becomes deficient (SMITH and LONERAGAN, 1997).

Rates of sulphate uptake are tightly controlled, increasing by as much as 15-fold within 48 hours after removal of the external sulphate supply, and returning to the low rate of sulphur-adequate plants within 24 hours of resupplying sulphate to sulphur starved plants (CLARKSON *et al.,* 1983). The uptake of potassium and phosphorus is also subject to feedback regulatory mechanisms. SMITH and LONERAGAN (1997) indicate that the uptake of nitrate via a constitutive transporter is required to trigger induction of a regulated nitrate transporter and a nitrate reductase. Accumulation of nitrogenous compounds then serves to repress excessive nitrate uptake and reduction.

2.3.6 Partitioning and remobilisation of nutrients

During absorption by roots and translocation in the xylem sap to shoots, nutrients may be transferred to phloem sap or withdrawn and deposited in root, stem and leaf cells (PATE, 1975).

SMITH and LONERAGAN (1997) state that nutrients absorbed in excess of the plant's immediate requirements are held at various sites within the plant or lost by guttation from shoots, excretion from roots or by death and abscission of plant parts. For each nutrient, the pattern of distribution and the rate and extent of cycling and remobilisation varies widely with the nutrient, environmental conditions, and plant nutrient status, species, and stage of development.

NAMBIAR and FIFE (1991) found whilst experimenting with conifers that nutrient uptake and growth rate are primary determinants of the amount and rate of retranslocation of nutrients rather than only senescence as previously thought. They found that rapid growth associated with high soil fertility and rapid nutrient uptake increased the retranslocation of nutrients and

there was competition among parts of the shoots for internal nutrient reserves. SWITZER and NELSON (1972) estimated that in a 20-year-old plantation of Loblolly pine, internal recycling accounts for 20-30% of the trees requirements for nitrogen, phosphorus and potassium, about 16% for magnesium and sulphur, and 7% for calcium. With such massive movement of nutrients in plants, it is not surprising that partitioning and remobilisation play central roles in relations between nutrient concentration and plant growth. An understanding of how they influence nutrient levels in plant tissues greatly enhances the interpretation of plant analyses (SMITH and LONERAGAN 1997).

2.3.7 Remobilisation

Nutrients deposited in tissues and organs may be remobilized and transported to other plant parts. Remobilisation from older to younger parts during plant development and nutrient stress may lead to rapid changes in concentrations of nutrients in particular leaves or organs and impacts strongly on the expression of deficiency symptoms, and on the relationship of plant nutrient concentrations to growth and yield (CLARKSON *et al.*, 1983).

According to SMITH and LONERAGAN (1997), whilst all nutrients move readily in the xylem, they vary widely in the extent to which they move in the phloem. Nutrients have been characterised as having high, low or intermediate phloem mobility on the basis of several criteria including nutrient concentrations in the phloem sap, movement of isotopes, development of deficiency symptoms and changing the nutrient content of organs.

DELL (1996) states that an understanding of nutrient function and behaviour in the plant is a prerequisite to diagnosing nutrient deficiencies by visual symptoms. The position within the shoot where symptoms first appear, largely depends on the mobility of the nutrient within the phloem. This is due to the ability of older leaves to redistribute a nutrient to sink areas, such as expanding leaves, root tips and young fruits, when the external supply is limiting. In agricultural species it is conventional to group the essential plant nutrients into three groups:

- **Phloem-mobile nutrients** - Nutrients that are exported from leaves under nutrient deficiency or as leaves senesce.
- **Phloem-immobile nutrients** - Nutrients that are retained in the leaves.
- **Variable phloem-mobile nutrients** - Nutrients that may be exported from leaves under particular conditions.

LONERAGAN *et al.* (1976) support the grouping for the diagnosis and prediction of nutrient deficiencies. They note that it is based on the internal movement of nutrients from older leaves to younger leaves and other developing organs during the onset of deficiencies. Whilst no detailed studies on nutrient redistribution in eucalypts exist, observations on the development of symptoms in a range of one to three-year-old eucalypts indicate a standard pattern. Data on differences in nutrient concentrations between green and senescent eucalypt leaves support these groupings (DELL, 1996).

2.3.7.1 Phloem-mobile nutrients

Nitrogen, phosphorus and potassium are present in high concentrations in the phloem sap and cycle rapidly throughout the plant. As their supply becomes limiting, young leaves and other developing organs retain the cycling nutrients at the expense of older leaves in which their contents decline (MARSCHNER, 1999). Consequently, during the development of their deficiencies, the concentrations of these nutrients remain high in young leaves while they decline in older leaves.

The decline in the content of these nutrients may occur in relatively young leaves, independently of senescence. NAMBIAR and FIFE (1991) have shown that relatively young needles of conifers can lose appreciable amounts of nitrogen and phosphorus to new shoots during periods of rapid shoot growth. Further losses occur during senescence. In nitrogen deficient plants, the deficiency itself induces premature senescence with protein hydrolysis and chloroplast disintegration resulting in early and rapid loss of nitrogen from older leaves.

PATE (1975) states that magnesium behaves in some respects like the phloem-mobile elements and its concentration in phloem sap is high relative to calcium. According to SMITH and LONERAGAN (1997), magnesium is retranslocated from green leaves to young organs and magnesium deficiency symptoms develop first in old leaves.

2.3.7.2 Phloem-immobile nutrients

Calcium, boron, manganese and iron, are characterised by their failure to move rapidly enough from leaves in which they have been deposited to prevent the onset of their deficiencies in young leaves, roots, or other developing organs when their supply from the roots becomes inadequate (SMITH and LONERAGAN, 1997). Leaves may lose appreciable quantities of these nutrients in guttation fluid and leachate but little, if any, moves to other plant organs. During the development of their deficiencies, concentrations of these nutrients remain high in

older leaves even when senescent, whilst they decline rapidly in young leaves. To avoid deficiencies of nutrients in this group, plants must have a continuous supply in the external medium or be able to tap reserves stored in organs other than leaves. Roots, young leaves, buds, seeds and fruits may fail to develop normally when the external supply of these nutrients becomes inadequate, even in plants with high total contents thereof. Such cases can lead to misinterpretation of plant analysis results unless special sampling procedures are adopted (SMITH and LONERAGAN, 1997).

The failure of calcium to move from old leaves has been attributed to its low concentration in the phloem sap (PATE, 1975). Calcium deficiency thus develops in young leaves and other organs and is largely independent of the total amount of calcium in the plant, in many cases developing as soon as the external supply of nutrient becomes inadequate, regardless of how much excess calcium may be stored in older leaves (SMITH and LONERAGAN, 1997).

According to SMITH and LONERAGAN (1997), boron resembles calcium in its failure to move from older leaves to developing organs and its requirement in the immediate environment for root growth. OERTLI (1993) found that tomato plants transferred from culture solutions with high boron concentrations to solutions without boron, rapidly developed boron deficiency symptoms in their young leaves but lost none of their boron from old leaves with high boron concentrations and symptoms of boron toxicity. However, whilst excess boron in old tomato leaves is not exported in the phloem, appreciable amounts may be lost in guttation fluid and washed from leaves. There is no proof that this is borne out in woody species. HU and BROWN (1994) state that boron retained by plant cells appears to be confined to and tightly bound to pectic compounds of the cell wall.

NABLE and LONERAGAN (1984) suggest that manganese resembles calcium in its failure to move from old leaves to developing organs and in the poor growth of roots in environments from which it is absent. In addition, applications of manganese are effective only in the short term. SHELP (1993) notes that unlike calcium, manganese can be present in phloem sap at concentrations which are high relative to tissue requirements.

2.3.7.3 Variable phloem-mobile nutrients

Sulphur, copper and zinc remain in old green leaves as their supply becomes deficient but move rapidly from the leaves during senescence. As a result, the concentrations of these nutrients in old green leaves do not respond as rapidly to declining nutrient supply as they do in young leaves, which are the first to show deficiency symptoms. Experiments have shown that even plants which accumulated inorganic sulphate in the vacuoles of their old green leaves before transfer to sulphate-free solutions, exported it too slowly to prevent sulphur deficiency developing in their young leaves (CLARKSON *et al.*, 1983).

The retention in and movement of copper, zinc and organic sulphur from leaves is closely related to the movement of nitrogen. Unlike nitrogen deficiency, deficiencies of these nutrients do not trigger senescence and may even delay it (SMITH and LONERAGAN, 1997).

2.4 The nutrient physiology of eucalypts

2.4.1 Nutrients and their role in eucalypts

According to DELL and ROBINSON (1993), the mobility of nutrients in the phloem of eucalypt seedlings is not greatly different from that in crop plants that have been extensively studied. In their study of *E. maculata*, DELL and ROBINSON (1993) found that the position in the shoot where deficiency symptoms first occurred and the progression in symptoms over time, are recorded as being similar to those for a range of woody crop plants. Deficiency symptoms of nitrogen, phosphorus, potassium and magnesium first appeared in mature leaves which is indicative that these macro-nutrients are readily retranslocated to younger tissues during the onset of deficiency. By contrast, calcium (which is not transported in the phloem) and sulphur (which may have variable phloem-mobility) deficiency symptoms first appeared in the shoot apex and young leaves. Iron was the least phloem-mobile micro-nutrient since symptoms progressed from young leaves to old leaves, whereas deficiency symptoms for manganese and zinc did not affect the shoot apex until growth was severely impaired.

In copper deficient plants, early stem bleeding coincided with reduced lignification of the secondary xylem and this preceded the onset of foliar symptoms. This suggests that lignification of the wood is very sensitive to the copper status of the plant and may be a very useful anatomical character to diagnose copper deficiency in eucalypt seedlings (DELL and ROBINSON, 1993).

The appearance of symptoms indicating nutrient deficiency is often related to the function of the nutrient in the plant, an insufficient supply leading to impairment of cellular function. For example, deficiency in nutrients such as iron and manganese that are essential for chloroplast formation and function causes chlorosis (yellowing) in leaves. In contrast, deficiencies of nutrients such as zinc and calcium that are essential for cell ontogeny are manifested in meristematic regions and result in malformed organs (DELL, 1996).

DELL (1996) states that eucalypts in their native landscape rarely exhibit symptoms of nutrient disorders. However, in plantations and forest nurseries, a range of macro- and micro-nutrient deficiencies are commonly encountered.

Five categories of deficiency symptoms have been observed :

1) Leaf chlorosis
2) Leaf necrosis
3) Leaf reddening due to the accumulation of anthocyanins
4) Leaf deformation
5) Dieback of shoot tips.

Procedures for diagnosing the nutrient status of eucalypts can be based on external symptoms, tissue histochemistry, enzyme activities and nutrient concentrations in organs such as stems and leaves. According to BOULD (1983), the two most widely used approaches are diagnosis by symptoms and by plant analysis.

2.4.2 The functions of certain nutrients in eucalypts

2.4.2.1 The Macro-nutrients

2.4.2.1.1 Nitrogen (N)

According to DELL (1996), nitrogen is a constituent of amino acids, proteins, nucleic acids, chlorophyll, many co-enzymes, ATP, alkaloids and many other classes of compounds. It is also the structural component of cell walls. The major effect of nitrogen deficiency is impaired protein synthesis and plant growth. An early symptom of deficiency is leaf chlorosis due to reduced chlorophyll formation. Carbohydrates may accumulate as they are not utilised for protein synthesis. Nitrogen is readily mobile in the phloem. In young seedlings, nitrogen which is surplus to the demand of growing tissues accumulates in mature, non-senescent leaves.

The efficient use of nitrogen by a leaf in accumulating carbon depends on how the nitrogen is partitioned to compounds involved in photosynthesis versus other substances. The efficiency of nitrogen utilisation in carbon assimilation is usually greater at higher nitrogen concentrations, but varies among species. The efficient use of nitrogen by a plant for biomass accumulation will also depend on the proportion partitioned to different organs and on rates of respiration. The efficient use of water by leaves in accumulating carbon depends on rates of carbon assimilation and transpiration. Leaf conductance is often positively related to foliar nitrogen concentration, but quantitatively, the relationship varies with species (SHERIFF and NAMBIAR, 1991).

Low pH is favourable to the accumulation of ammonium in the soil and some work suggests that uptake of ammonium is preferred to nitrate (ADAMS and ATTIWILL, 1982). VALE *et al.* (1984) grew plants of an *E. alba* clone for two or 48 hours in nutrient solution containing aluminium and the rate of depletion of ammonium was greater than that of nitrate from a solution in which ammonium and nitrate were present in equimolar concentrations. Pre-treatment with aluminium tended to increase uptake of both forms of nitrogen. The increase in nitrate uptake may be due to a reduction in the negative potential caused by the adsorption of aluminium in the free space in the roots or a reduction in the permeability of the plasmalemma.

The growth of *E. grandis* seedlings for 90 days in nutrient solution with different NH_4^+ : NO_3^- ratios was greater when both sources of nitrogen were supplied in equal concentrations (LOCATELLI, 1984). However, roots became darker and grew slower as the proportion of nitrate-nitrogen increased. This could be a response to nitrate uptake where organic acids are exuded and may then accumulate, along with iron or aluminium, at the root surface. SHEDLEY *et al.* (1995) note that in *E. globulus*, seedlings responded to higher rates of ammonium and ammonium nitrate than to nitrate. The maximum shoot dry weight (DW) for nitrate-fed plants and ammonium nitrate-fed plants was 51% and 84% respectively of ammonium-fed plants. Table 2.19 summarises the responses of *E.globulus* to varying forms of nitrogen.

Table 2.19 Total nitrogen concentration in the YFEL (youngest fully expanded leaf) for *E. globulus* seedlings grown in a glass house with three different N sources

	Ammonium	Ammonium nitrate	Nitrate	Symptoms
Severely deficient	< 1.4.%	< 1.4.%	< 1.4.%	Stunted, general chlorosis
Deficient	1.5-2.8%	1.5-2.5%	1.5-2.3%	Reduced shoot growth, interveinal chlorosis
Adequate	2.9-4.7%	2.6-4.3%	2.4-3.3%	Blue green leaves
Toxic	>4.7%	>4.3%	>3.35	Ammonium and ammonium nitrate-excessive, leaf expansion and wilting. Nitrate-stunted growth, xeromorphy and leaf tip necrosis

(SHEDLEY *et al.*, 1995)

The preference of *E. globulus* seedlings for ammonium-N over nitrate-N has been observed in other eucalypt seedling studies, and this may be due to higher net uptake rates of ammonium (two to five times) than for nitrate or to pH effects. Ammonium nutrition can also enhance the uptake of phosphorus and sulphate. It has been noted in *E. diversicolor* seedlings that the

preference for ammonium disappeared as seedlings became mycorrhizal. The accumulation of nitrate in the stem of nitrate-fed seedlings suggests an inefficiency in nitrate reduction within the plant rather than impaired uptake by the root (SHEDLEY *et al.*,1995).

FERREIRA (1986) studied the effect of the addition of nitrate, phosphate and sulphate on the growth of *E. grandis* seedlings using a split-root (root system divided between two pots) technique. The three anions were supplied in various combinations to the two pots and all other nutrients were maintained in adequate concentrations. The separation of one anion from the other two caused a decrease in plant shoot growth. Best growth was obtained when both pots were supplied with the three anions. Root growth was particularly improved in the pot to which nitrate had been applied. For any combination of the three anions, 82-91% of the total reduced nitrogen in xylem exudate was as glutamine (Table 2.20). However, when one anion was isolated from the other two, arginine was detected in significant concentrations, and was associated with low plant growth. This supports the hypothesis that there is a lack of carbon to assimilate the reduced nitrogen (DE BARROS and DE NOVAIS, 1996).

Table 2.20 Concentrations of amidic and soluble aminic nitrogen in dry leaves of *E. grandis* seedlings after 47 days of growth in different combinations of nitrate, phosphate and sulphate in nutrient solution in a split-root system. The hyphen in the treatments indicates the division between the two pots in which the root system was split. Other nutrients were present in equal amounts in all pots

Form of N	Treatments				
	½ NPS -½ NPS	NPS - 0	N - PS	P - NS	S - NP
		Concentration (%)			
Aspartic acid	5.7	7.1	5.7	5.7	4.2
Asparagine	2.3	2.4	1.8	2.5	1.4
Glutamic acid	7.9	9.2	7.7	10	6.4
Glutamine	63.2	60.8	37.5	46.5	23.6
Alanine	11.8	10.4	9.2	11.6	8.3
Serine	4.6	5.3	4.2	5.2	5
Arginine	-	-	29.1	13	46.9
Others	4.5	4.8	4.8	5.5	4.2

(FERREIRA, 1986)

COSTA (1986) also found that glutamine was the dominant form of nitrogen transported to shoots while arginine was dominant among free amino-nitrogen in leaves. The satisfactory growth of the plants in nitrate as the only source of nitrogen and the preferential uptake of ammonium shows a metabolic adaptation to diverse nutritional conditions. That there was no

nitrate in the xylem exudate of the plants in the work of FERREIRA (1986), despite all nitrogen being applied as nitrate, suggests that nitrate reductase activity in the roots is considerable.

2.4.2.1.2 Phosphorus (P)

Phosphorus is required in energy transfer through the formation of phosphate esters and energy rich phosphates. It is a structural element of nucleic acids, phospholipids and phospho-proteins, and is important for the regulation of a few key enzymes. Carbohydrate biochemistry and transport are particularly affected in deficient plants. Phosphorus readily moves from old to young leaves, and from inner bark to shoot tips. Excess phosphorus may accumulate as inorganic phosphate in the inner bark (DELL, 1996).

KIRSHBAUM and TOMPKINS (1990) state that plants receiving inadequate supplies of phosphorus grow poorly and show a variety of other physiological responses such as increases in the ratio of root to leaf dry weight and a reduction in specific leaf area.

KIRSHBAUM and TOMPKINS (1990) grew *E. grandis* seedlings in growth units in which plant roots were suspended in air, while continuously being sprayed with nutrient solution (aeroponic system). Phosphorus was added to the nutrient solution in five exponentially increasing amounts. Plant growth rates and photosynthetic performance was compared across all treatments. Carbon assimilation rates ranged from 11.7 $\mu mol^2/s$ for plants with lowest phosphorus status to 23.1 $\mu mol^2/s$ for plants with highest phosphorus status. Intercellular partial pressures of CO_2 decreased from 260 μbar for plants with lowest, to 220 μbar with highest phosphorus status. Leaves in all treatments showed a decrease in assimilation rate at intercellular partial pressures of CO_2 above 600 μbar. There was no consistent correlation between the extent of that decrease and the phosphorus status of leaves. The photosynthetic measurements showed that CO_2 assimilation rate, together with relative leaf growth rate, was one of the processes most sensitive to phosphorus nutrition.

DE BARROS and DE NOVAIS (1996) state that the critical concentration of available phosphorus (e.g. Mehlich-1 available-phosphorus) falls exponentially with plant age, from 37 µg/g for 90 day old seedlings to 7 µg/g for 150 day old seedlings. For six-year-old trees, 4 µg/g of phosphorus is sufficient to maintain incremental growth at the rate of 60 m^3/ha per year in a soil containing 50% clay.

The relationship between soil acidity and preferential uptake of ammonium holds true for phosphorus nutrition. VALE *et al.* (1984) examined the effect of the form of the available nitrogen on the kinetics of phosphorus uptake by *E. alba* seedlings. Seedlings grown in nutrient solution with ammonium as the only nitrogen source exhibited a greater V_{max} - P (maximum rate of absorption) and lower K_m (Michaelis constant : represents that ionic concentration giving half of the maximum rate of absorption) than those grown in a nitrate solution. Plants supplied with ammonium absorbed 55% more phosphorus than plants supplied with nitrate. It can be concluded that in soils of low pH, nitrogen uptake will be aided by the predominance of the ammonium form. This will, in turn, enhance phosphorus uptake from the dilute soil solutions (DE BARROS and DE NOVAIS, 1996).

According to O'CONNELL and GROVE (1984), the measurement of phosphatase activity is a useful biochemical test of phosphorus status and a possible diagnostic alternative to foliar analysis. Phosphatase activity generally increases with increasing phosphorus deficiency. In most plants, phosphatase activity increases from two to four fold as phosphorus supply is reduced. Greater enzyme activity is thought to be due to increased synthesis of phosphatase under phosphorus deficient conditions. In *E. diversicolor* the enzyme activity was highest for low levels of added phosphorus and decreased with increasing additions of phosphorus. This increase in phosphatase activity with phosphorus deficiency relative to activity at the highest level of applied phosphorus was greatest for stems (4.3-fold), intermediate for mature and partly expanded leaves (3.7-fold) and lowest for shoot tips (2.7-fold). Phosphatase activity in *E. diversicolor* seedlings was highest in shoot tips and lowest in old stems. Phosphorus concentrations in old stems reflect phosphorus supply over a wide range of added phosphorus, indicating that old stem tissue may act as a sink for excess phosphorus taken up by the plant. The close relationship of phosphatase activity and phosphorus concentration, suggest that to assess plant phosphorus status, old stems may be appropriate tissue for both biochemical enzyme assay and conventional nutrient analysis. Phosphorus concentrations in mature leaves are simply related to phosphorus supply indicating that leaves may be less useful as a diagnostic tissue than old stem tissue.

Although reduced soil phosphorus levels can significantly increase enzyme activity in a number of plant tissues of *E. diversicolor* seedlings, the activity may also be influenced by changes in soil nitrogen supply and plant protein levels. At high levels of added phosphorus, increased plant growth and microbial immobilisation of nitrogen in the soil may result in reduced supply of mineral nitrogen for plant uptake. Consequently leaf and stem tissue nitrogen concentrations

and proteins, can decrease with increasing added phosphorus (O'CONNELL and GROVE, 1984).

WALLACE *et al.* (1986) report that phosphorus concentrations in shoots and, to a lesser extent roots, were high when zinc was deficient. With successive increases in zinc concentrations up to 400 µg Zn/kg dry soil, a trend of reducing phosphorus was evident, despite an increase in phosphorus content. They attributed the depression in phosphorus concentration with zinc application to the promotion of growth by zinc, which diluted the available phosphorus in the plants.

LACEY *et al.* (1966) discovered that seedlings of *E. grandis* grown in hydroponic culture under luxury phosphorus supply contained 0.32% phosphorus in both leaves and roots, whilst O'CONNELL *et al.* (1978) reported that levels for mature leaves of field grown eucalypts are usually about 0.5% phosphorus. According to WALLACE *et al.* (1986), the application of zinc alleviated symptoms of excess phosphorus concentrations in mature leaves. A zinc deficiency enhanced phosphorus absorption and transport, at high phosphorus supply, but it also appeared to affect phosphorus translocation and remobilisation.

2.4.2.1.3 Potassium (K)

Potassium functions in the stabilisation of pH and osmoregulation, is required for the synthesis of proteins and carbohydrates, is an activator of numerous enzymes and has a pivotal role in the control of stomatal aperture. In deficient plants, protein synthesis and photosynthesis are impaired, and localised cell death may occur. Potassium moves freely in the phloem, so it is readily exported from old leaves (DELL, 1996).

2.4.2.1.4 Calcium (Ca)

DELL (1996) states that most calcium in a plant is localised in the cell vacuoles (often as crystals of calcium oxalate) and in the cell walls, where it is associated with pectin in the middle-lamella. PEREIRA *et al.* (1996) note that calcium accumulates in the bark partly in the form of calcium oxalate crystals to a concentration that reaches 20 times that found in the wood. This calcium accumulation seems to be largely opportunistic and dependent on culture media.

Calcium is also required for membrane stability, and hence is essential for cell division. Calcium plays a role in osmoregulation as well as cation-anion balance. It is not transported in

the phloem and growing root tips and shoots are vulnerable to its deficiency. Root tips require calcium in the rhizosphere for normal growth (DELL, 1996).

MENGEL and KIRBY (1982) state that the uptake of calcium is restricted to young root tips. Calcium transport from the cortex to the stele is through the apoplastic pathway in the still unsuberised root zone in which the casparian strip has not yet formed. The use of discing for weed control is common practice in many areas of eucalypt afforestation and the effect of cutting root systems is frequently questioned. However, DE BARROS and DE NOVAIS (1996) suggest that calcium uptake may be enhanced since the trimming of roots will stimulate the growth of new roots.

ANDERSON (1982) reported that the inability of *E. obliqua* seedlings to grow on calcareous soil is due to either a low uptake of iron or an inactivation of iron within the plant. Both calcium and phosphorus affect iron availability and activity. The chlorosis that develops in *E. obliqua* leaves is as a result of the inactivation of iron at high external levels of calcium. Foliar analysis of severely chlorotic seedlings suggest that high levels of calcium (500 µg Ca/ml) may have a detrimental effect on iron absorption and metabolism.

Seedlings of *E. obliqua* showed a increase in growth from 5 to 100 µg Ca/ml followed by a marked decrease when the concentration was raised to 500 µg Ca/ml. It has been suggested that balanced potassium : calcium ratios are required for the normal metabolism of green plants and that high levels of calcium can have an inhibitory effect on the reactions of respiration and intermediary metabolism (ANDERSON, 1982).

2.4.2.1.5 Magnesium (Mg)

A major function of magnesium is as a co-ordinated metal in chlorophyll. It is also required for protein synthesis, the activation of many enzymes, and the regulation of cellular pH and cation-anion balance. Magnesium is retranslocated in the phloem from old leaves (DELL, 1996).

In comparing the response of *Nothofagus* species to differing concentrations of calcium, potassium and magnesium, SUN *et al.* (2001) report that magnesium appeared to affect seedling growth the most. Decreasing rates of magnesium supply reduced the growth rates of both shoots and roots and resulted in leaf chlorosis and senescence.

2.4.2.1.6 Sulphur (S)

FERREIRA (1986) states that sulphur uptake of *E. grandis* is highly dependent on the presence of nitrate and phosphate. According to DELL (1996), it is a constituent of the amino acids cysteine and methionine, hence protein synthesis is impaired in sulphur deficient plants. It is also required for the production of thiamine, co-enzyme-A and sulfolipids. A decline in the sulphur chlorophyll content of sulphur deficient plants leads to leaf chlorosis. Sulphur is not very mobile in the phloem and therefore symptoms of deficiency first appear in young leaves.

2.4.2.2 The micro-nutrients

2.4.2.2.1 Iron (Fe)

Many of the reactions associated with iron, such as the redox reactions of photosynthesis and respiration, are linked to its reversible oxidation states (Fe^{2+}, Fe^{3+}). Iron is bound to haemoproteins (e.g. the cytochromes and peroxidases) and is a component of a few Fe-S proteins (e.g. ferredoxin). Since iron is required for the synthesis of chlorophyll and does not move out of old leaves, chlorosis of young leaves is an early symptom of iron deficiency (DELL, 1996).

Under controlled conditions, the growth of *E. grandis* seedlings in four sandy soils (<15 % clay) showed no response to increasing amounts of manganese or iron (both at levels of 0-320 µg/g of soil) (NOVAIS *et al.*, 1990). Manganese and iron absorption by *E. grandis* may depend on preferential uptake of ammonium and a decline in pH (DE BARROS and DE NOVAIS, 1996).

In a test carried out by NOVAIS *et al.* (1990), the toxicity of iron and manganese on *E. grandis* seedlings was tested in nutrient solution (pH adjusted daily to 4.7), with increasing rates of both nutrients (5-320 µg/mL of manganese and 20-400 µg/mL of iron). Whereas shoot and root growth was maintained at up to 100 µg/mL of manganese, growth decreased markedly above 40 µg/mL of iron. At 160 and 320 µg/mL of iron the root system became discoloured and dark, and the leaves died after the first day of treatment. Seedling growth was impaired when the concentration of iron in the tissues reached 1035 µg/g and for manganese when the concentration reached 11000 µg/g.

2.4.2.2.2 Manganese (Mn)

Manganese is required for the splitting of water by light in Photosystem II and the Mn-protein, superoxide dimutase, and probably has a role in protecting chloroplast membranes. Manganese has properties similar to magnesium and can substitute for magnesium in some

enzyme systems. It may also be involved in the regulation of auxin levels through auxin oxidases. Symptoms of deficiency develop in recently expanded and expanding leaves and it is likely that manganese movement in the phloem is limited from old leaves to shoot the apex (DELL, 1996).

2.4.2.2.3 Zinc (Zn)

DELL (1996) notes that zinc is important for stem elongation and photosynthesis. It is a constituent of a number of enzymes, including carbonic anhydrase and alcohol dehydrogenase, and is required for the activity of many other enzymes. SANDMANN and BÖGER (1983) found that the activity of the metalloenzyme carbonic anhydrase, which catalyses the reversible hydration of carbon dioxide, is strongly repressed in young, expanded leaves of zinc deficient eucalypt seedlings. As the greatest range in enzyme activity occurs within the range of adequate supply, DELL and WILSON (1989) conclude that foliar zinc analysis may be a better diagnostic tool than leaf carbonic anhydrase activity. The carbonic anhydrase assay has not yet been evaluated for zinc deficient eucalypts in the field.

DELL (1996) indicates that the characteristic reduction in leaf size and shortening of internodes in zinc-deficient trees is probably related to the requirement of zinc for the synthesis of auxin. Phosphorus uptake may increase under zinc deficiency and symptoms of zinc deficiency may be confounded by those of phosphorus toxicity in fully expanded leaves. As it is not readily redistributed from old leaves, symptoms of deficiency first appear in expanding leaves. According to DELL and WILSON (1985), some of these deficiencies may be the result of changes in growth rates at the apical meristem. This is likely to be due to reduced IAA concentrations, since zinc is required for synthesis of tryptophan. As well as its role in auxin production, zinc plays an integral part in the activity of carbonic anhydrase and the carbonic anhydrase assay[22] may prove to be an excellent tool in determining zinc deficiency.

WALLACE *et al.* (1986) have found that in zinc-deficient *E. marginata*, salts accumulate at the guttation sites at the tips and margins of fully expanded leaves, which then die. They also noted a decrease in the height of seedlings, a decrease in the length of internodes on the main stem and the size of the leaves. Zinc deficiency had no effect on root morphology or colour. Within 28 days of sowing, symptoms of zinc deficiency were evident at concentration ranges of 0 to 67 µg Zn/kg dry soil. These started as bronzing on the adaxial surface of young leaves followed by a light chlorosis.

Experiments by DE BARROS and DE NOVAIS (1996) showed no response by a variety of eucalypt species to the application of micro-nutrients, irrespective of soil type or nutrient concentration. *E. grandis* seedlings cultivated in a glass house did not respond to increasing amounts of boron and zinc when grown in any of five, infertile savannah soils. In a similar trial, growth was again unaffected by the application of zinc (0.0 - 4.8 µg/g of soil) although there was some accumulation of zinc in the foliage when large amounts were added.

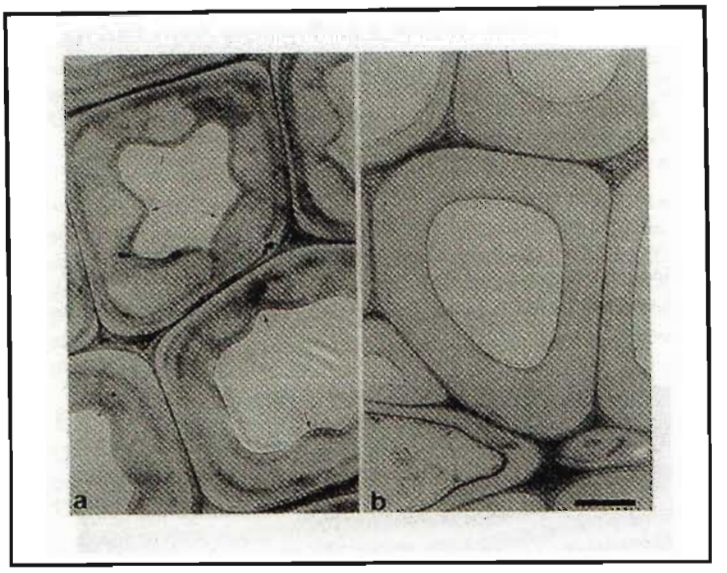
2.4.2.2.4 Copper (Cu)

According to MARSCHNER (1999), most of the function of copper in plants depends upon the participation of enzymatically bound copper in redox reactions. One group of these copper metallo-enzymes is polyphenol oxidase which contains both laccase and catechol oxidase. One possible function of catechol oxidase is the synthesis of o-diphenols but the physiological functions of these enzymes is yet to be fully established. However, many workers claim that these enzymes are involved in the biosynthesis of lignin and other compounds.

DELL (1996) states that much of the copper in leaves is located in chloroplasts where it is bound to plastocyanin, a major constituent of the electron transport chain of Photosystem I. Photosynthesis is therefore, reduced in copper deficient plants. Symptoms first appear in meristematic areas, and this suggests that copper is immobile in the phloem of deficient trees. It is also a constituent of a number of Cu-metalloenzymes such as cytochrome oxidase and phenolase; thus it is essential for normal wood development. HANSON and HAVIR (1979) indicate that phenolase has two distinct activities: an oxidase function and a mono-oxygenase reaction.

BUSSLER (1981) found that the reactions catalysed by phenolase are involved in the synthesis of lignin and the production of alkaloids. Copper deficiency results in decreased phenolase activity and impaired cell wall lignification. DELL (1996) has found that in addition to decreased cell wall lignification, copper deficiency can also affect the development of cell wall layers (Figure 2.6). DELL (1994) has recorded that in *E. maculata*, lignification of wood was strongly depressed when copper concentrations fell below 1.5 µg/g DW and BUSSLER (1981) observed that in herbaceous plants, re-lignification does not occur when copper is supplied to copper-starved plants.

Figure 2.6 Transverse sections of xylem fibres of *E. maculata* under the transmission electron microscope. a) Fibres from copper deficient wood with convoluted lumen borders and unlignified areas in the secondary wall. b) Normal lignified fibres from wood with adequate copper. Scale = 10 μm



(Dell, 1996)

2.4.2.2.5 Boron (B)

The role of boron cell metabolism is poorly understood. It appears to be essential for cell division, cell growth and possibly membrane function. Like calcium, boron is not loaded into the phloem. Growth of shoot and root tips is seriously impaired in boron-deficient plants (DELL, 1996).

2.4.2.2.6 Molybdenum (Mo)

Molybdenum is required for nitrogen fixation in root nodules as the metal is a constituent of nitrogenase. In plants not reliant on nitrogen fixation, molybdenum is required for the function of nitrate reductase and is only essential for eucalypts where inorganic nitrogen is taken up as nitrate (DELL, 1996).

2.4.2.2.7 Silicon (Si)

GILLMAN and ZLESAK (2000) have found that leaf retention is an important consideration when rooting vegetative cuttings. Softwood cuttings benefit from carbohydrates stored in leaves as well as from auxins produced therein. Methods that increase leaf retention on softwood cuttings have the potential to stimulate rooting. Additions of sodium silicate to nutrient solutions affect the disease resistance of plants and may enhance the leaf retention of cuttings

by inhibiting fungal infection and development. Silicon compounds are applied to plants by adding them to the nutrient solution contacting the root zone. BOWEN *et al.* (1992) state that foliar applications of silicon compounds may be superior to root zone application for disease control.

In roses, silicon treatments increased rooting and improved leaf retention at application rates of 50 and 100 mg/L (GILLMAN and ZLESAK, 2000). Greater leaflet retention probably increased available carbohydrates and leaf-produced auxins resulting in increased rooting (HARTMANN *et al.*, 1990). Higher concentrations of silicon (150 mg/L) were not as effective in reducing leaf loss, probably as a result of silicon build up on the leaves and an increase in water pH.

BUGBEE (1996) reported that although silicon has not been recognised as an essential element for higher plants, its beneficial effects have been shown in many plants. Silicon is abundant in all field grown plants, but it is not present in most hydroponic solutions. The beneficial effects of silicon (Si) are two-fold: 1) it protects against insect and disease attack and 2) it protects against toxicity of metals .

2.4.2.2.8 Aluminium

HUANG and BACHELARD (1993) state that although aluminium is commonly found in plant tissues and may stimulate growth, it is not considered to be an essential element and excess concentrations can be toxic. Aluminium toxicity is closely associated with soil acidity and many low fertility forestry soils are associated with high aluminium and low calcium. It has been hypothesised that high aluminium content in soils combined with strong acidity might contribute to differences in tree species in growth, root structure and cation uptake. In *E. mannifera*, aluminium in a culture solution increased the growth of roots and shoots of seedlings up to levels of 2.222 µ*M*. Root and shoot concentrations of potassium increased with increasing aluminium, whilst calcium and magnesium concentrations decreased and manganese concentrations were unaffected. Calcium and magnesium concentrations in tissue were two to three times higher in the eucalypt than in pine seedlings (*Pinus radiata*) at all levels of aluminium added, due to greater uptake of these elements by the eucalypt. At higher concentrations (2.222 µ*M*) of aluminium in the culture medium, shoot aluminium concentrations were lower in the eucalypt than in the pine seedlings due to the greater proportion of aluminium held in the eucalypt roots. The differences between seedlings in terms of root growth and tissue cation concentrations may help explain the ability of eucalypt species to

maintain vigorous growth in acid media high in aluminium and low in calcium and phosphorus, where the growth of pines has failed.

According to MULETTE (1974), aluminium tolerance is conferred upon plants able to produce organic acids, particularly citric and oxalic, in their roots where the acids form complexes with the aluminium ions, thereby preventing toxicity. Tolerant plants are known to accumulate aluminium in their tissues including roots, stems and leaves and aluminium is complexed as succinate and oxalate. However, the only available information for eucalypts shows that *E. gummifera* does not accumulate aluminium in its leaves.

Low concentrations of aluminium may even prove highly beneficial as soluble aluminium (low concentrations) can stimulate the uptake of phosphorus into both shoots and roots of tolerant legume species. It is thought that in root cell walls, aluminium can be adsorbed onto the free carboxyl groups, which in turn cause hydrolysis of the $Al(OH)^{2+}$ ions, precipitating $Al(OH)_3$ onto the root surface. This precipitate is thought to fix the phosphorus in an organic form onto the root surface, limiting normal metabolic uptake in the root cells (MULETTE, 1974).

According to MULETTE (1974), the conflict between the stimulative and inhibitory effects of aluminium on phosphorus uptake into the plant may in part be resolved, if at low concentrations, the aluminium ions block sufficient of the negatively charged sites of the cell wall material to facilitate entry of the large negatively charged ions into the cell. At higher concentrations of aluminium, the negative sites are super-saturated. Precipitation of the aluminium and the subsequent binding of phosphorus then follows.

The major deficiencies are summarised in Table 2.21.

Table 2.21 Key to deficiency symptoms in *Eucalyptus*. An accepted form of determining nutrient deficiencies is through a visual assessment and comparison to a known key as shown in this summary

A1 Symptoms appear first or are more severe on old leaves

B1 Leaf colouration is even over whole leaf

C1 Leaves pale green to yellow, small reddish spots may develop secondarily ..... Nitrogen

C2 Leaves green with reddish blotches or leaves uniformly purple to red ..... Phosphorus

B2 Leaf colouration forms a pattern

C1 Leaves with marked interveinal chlorosis ..... Magnesium

C2 Leaves with scorched margins or interveinal necrosis, sometimes preceded by marginal chlorosis ..... Potassium

A2 Symptoms appear first or are more severe on expanding leaves

B1 Dieback present at shoot apex

C1 Nodes enlarged, proliferation and death of lateral shoots

D1 Leaves with corky abaxial veins, or apical chlorosis, or malformed with incomplete leaves..... Boron

D2 Leaves with irregular or undulate margins, some interveinal chlorosis ..... Copper

C2 Nodes normal, leaves buckled due to impaired marginal growth ..... Calcium

B2 No dieback at shoot apex

C1 Leaves normal size

D1 Leaves pale green to yellow ..... Sulphur

D2 Leaves yellow with green leaves..... Iron

D3 Leaves with marginal or mottled chlorosis, small necrotic bleached or brown spots may appear ..... Manganese

(DELL, 1996)

2.4.3 Nutrient interactions in eucalypts

The complexity of nutrient physiology must be understood in the light of numerous and multiple interactions, many of which we know very little about. No single nutrient operates in isolation, there is a constant interaction of elements that activate and drive multiple biochemical pathways. This section is a brief and simplified summary of a few of the more understood and described processes.

TURNER and LAMBERT (1986) reported that when one nutrient is involved in the assimilation or metabolism of another nutrient, the symptoms may not clearly differentiate the causal element. For example, nitrogen and sulphur are biochemically related in plant proteins. Because there is no inorganic nitrogen in tree foliage, there is a constant ratio between organic (and total) nitrogen and organic sulphur, and the rate of sulphur accumulation. In many forest species, protein formation is limited by the amount of sulphur available, and the symptoms of nitrogen and sulphur deficiency are very similar.

WILL (1961) grew three eucalypt species (*E. botryoides, E. saligna* and *E. pilularis*) in perlite with either a full supply of nutrient elements or with a low supply of nitrogen, phosphorus, potassium, magnesium or calcium given singly. Nitrogen and phosphorus deficiency resulted in the complete suppression of branching, potassium deficiency resulted in reduced internode lengths and promoted the production of first and second order branches. He also found that nitrogen deficiency resulted in chlorotic yellowish green foliage often with red blotching.

According to O'CONNELL and GROVE (1984), there is a significant interaction between added nitrogen and phosphorus on growth of Karri seedlings (*E. diversicolor*). A phosphorus deficiency resulted in bluish or purplish blotching giving the foliage a darker green appearance (Table 2.21). Potassium deficiency did not affect foliage colour, but resulted in smaller than normal leaf size, with buckled leaf margins. A deficiency of magnesium led to paler green lower leaves, often followed by premature leaf drop. Seedlings subjected to calcium deficiency succumbed with a sudden cessation of growth, followed by withering of shoots and then death. In *E. pilularis*, multiple axillary shoots were produced before the seedlings succumbed.

KNIGHT and NICHOLAS (1996) found that seedlings of *E. fastigata, E. regnans* and *E. saligna* grown for six months in perlite with a full supply of all nutrients, or with individual nutrients singly omitted, exhibited deficiency symptoms where nitrogen, phosphorus, potassium, magnesium, sulphur, calcium and boron were singly omitted from an otherwise complete supply of nutrients. Their results were similar to those described by WILL (1961). Omission of sulphur caused a reddening of youngest foliage and necrotic patches on older leaves on the main stem. Seedlings grown without boron grew normally for a few weeks, then lost the erectness of the stems due to the formation of constrictions near the root collar; older leaves of *E. saligna* and *E. fastigata* developed brown discoloured venation, through abnormal suberisation. Seedlings raised with a low supply of phosphorus, calcium or magnesium were particularly prone to leaf cast .

WILL (1961) found that seedlings grown with a low supply of potassium were more extensively branched than usual, with *E. saligna* being the most sensitive species. KNIGHT and NICHOLAS (1996) remarked that a reduced supply or omission of individual macro-nutrients had a significant effects on height growth and production of dry matter.

KNIGHT and NICHOLAS (1996) determined nutrient concentrations from total foliage collections. Concentrations of nitrogen and phosphorus in foliage samples were insensitive indicators of the supply of nitrogen and phosphorus, because a very low supply of either nutrient reduced dry matter production without causing any further reduction in concentration of that nutrient. Experiments by INGESTAD (1982), CROMER (1984) and CROMER *et al.* (1984) have proven that seedlings can remain healthy at relatively low concentrations of foliar nitrogen, provided that stress is prevented by supplying nitrogen continuously.

DE BARROS and DE NOVAIS (1996) reported that the correlation between nutrition of eucalypts in a glass house and those in the field is highly significant. High tolerance to aluminium, preferential absorption of ammonium in relation to nitrate and low critical concentrations of calcium, magnesium, and potassium in the soil have all been observed in a variety of eucalypt species in both the glass house and the field.

NEVES *et al.* (1982) evaluated the effects of aluminium on growth, and phosphorus and aluminium uptake by seedlings of different eucalypt species. Aluminium tolerance, as determined by shoot and root growth of four species, was assessed for seedlings grown in a nutrient solution at pH 4.5 for 100 days with increasing concentrations of aluminium (0, 3, 9, 27 µg/mL). The results were as follows:

Best Tolerance - *E. urophylla*
E. paniculata
E. grandis
Worst Tolerance - *E. cloeziana*

E. cloeziana translocated aluminium to the shoots at high external concentrations of aluminium, and phosphorus concentrations in roots and shoots of this species were the highest of all species tested.

MULETTE (1975) noted *E. gummifera* (Gaertn.) Hochr. seedlings grown in pure quartz culture showed an optimal growth response at 1 mg/L supplied as aluminium phosphate. It is hypothesised that in the soil of its natural habitat, aluminium present as insoluble compounds, is released as aluminium ions at low concentrations and plays an important role in the

mechanism of phosphorus uptake. These soils have been shown to have aluminium as the dominant cation.

NEGI and SHARMA (1996) surmise that nutrient concentrations in foliage from fertile sites are greater than those from infertile sites. Nutrient concentrations under luxury supply increased two-fold for nitrogen, phosphorus, potassium and magnesium in all species. Under deficient supply of nutrients, concentrations of most nutrients, particularly nitrogen and phosphorus, were significantly greater in *Eucalyptus* than in *Pinus patula* (Table 2.22).

Table 2.22 Nutrient concentrations in one-year-old seedlings of different species grown in a pot culture experiment with luxury (L) or deficient (D) supply of nutrients

Species		N	P	K	Ca	Mg
E. grandis	L	2.38	0.21	0.8	0.84	0.29
	D	1.15	0.09	0.4	0.56	0.12
E. globulus	L	2.1	0.27	1.05	1.5	0.3
	D	0.98	0.14	0.47	1.28	0.17
E. citriodora	L	0.98	0.16	1.23	0.92	0.22
	D	0.48	0.13	0.79	0.7	0.12
Pinus patula	L	1.36	0.11	0.8	1.6	0.19
	D	0.64	0.02	0.31	0.96	0.04

(NEGI and SHARMA, 1996)

2.4.4 The role of carbohydrates in rooting

HARTMANN *et al.* (1990) state that rooting is generally accepted as being a multiple-step process in which totipotent cells dedifferentiate, producing undifferentiated meristematic tissue which then produces cells that differentiate as root initials, which subsequently elongate and emerge as visible roots from the stem tissues. DICK and DEWAR (1992) note that under certain conditions, a cutting with root initials may fail to root because it runs into a carbohydrate deficit. The carbohydrate status of cuttings has been shown to affect the rooting ability of many horticultural species including chrysanthemum, avocado, raspberry and apples (HARTMAN *et al.*, 1990). Similar correlations between rooting and carbohydrate content have been found in forest tree species, including *Pinus banksiana* (HAISSIG, 1984) and *Pinus sylvestris* (HANSON *et al.*, 1978).

It is assumed that structural root growth is limited by the amount of root sugar. Sugar is produced by leaf photosynthesis and net starch mobilisation, and translocated to the root where it is utilised for structural growth. The transport and utilisation of sugars is driven by

concentration gradients across resistances between the structural compartments of the cutting (DICK and DEWAR, 1992).

The ability of cuttings to photosynthesise during the early stages of root development has been considered advantageous to rooting. Besides carbohydrate status, factors such as stock plant nutrition, water relations and hormonal influences, appear to also limit root formation (DICK and DEWAR, 1992).

In trials on birch (*Betula pubesecens* J.F. Ehrh.), WELANDER (1995) reported that a rooting temperature of 16 °C delayed the rate of root production compared with higher temperatures, but the final rooting percentage was the same over the range from 16 to 28 °C. Root branching also increased with temperature. At all temperatures, there was a large increase in sucrose content at the base of cuttings during rooting, whereas the concentration of non-translocated sugars remained constant. The carbohydrate content at the base of micro-propagated stock was three times higher than that at the base of cuttings from seed stock, but the higher carbohydrate concentration was not correlated with a higher rooting potential. The opportunity to manipulate the rooting potential of cuttings is generally much greater when favourable conditions are given to the stock plants rather than the cuttings taken from them. The mineral nutrient status of the stock plant affects rooting, a low nitrogen supply improves rooting and irradiance also influences the rooting ability of many plants.

Although irradiance has not always been associated with rooting potential, it has been used to change the carbohydrate content of cuttings. HAISSIG (1984) states that carbohydrates are needed for root initiation. However, the amount of carbohydrate required in the process is not known, and the relationship with root formation is unclear.

2.5 Foliar analysis as a management tool

2.5.1 Nutrient concentration trends

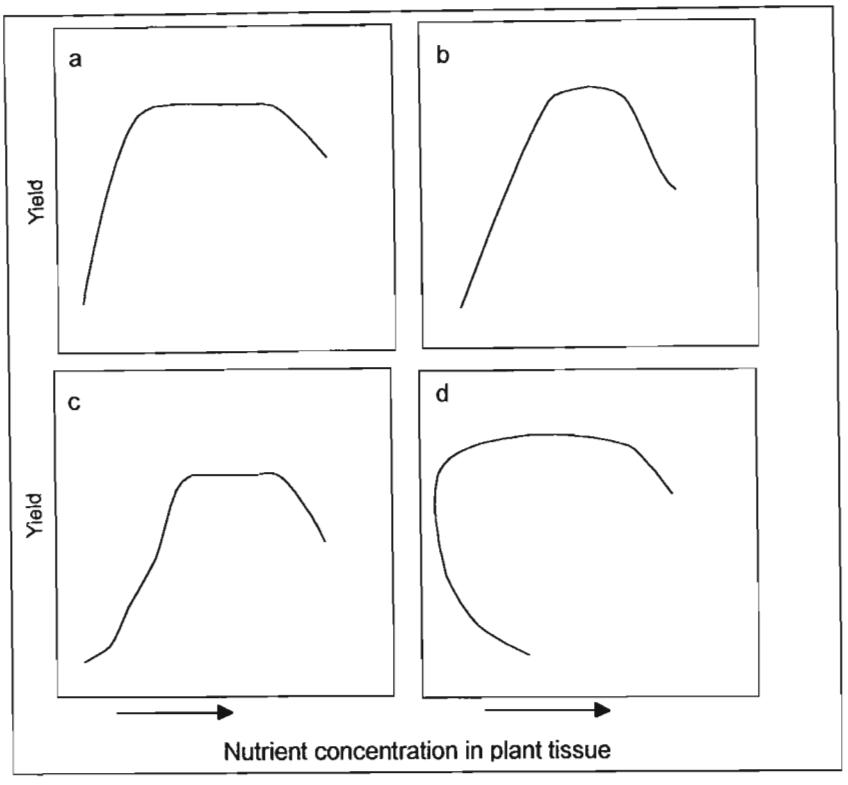
According to NEGI and SHARMA (1996), foliar concentrations of nutrients are important ecological attributes which have been used in studies of nutrient cycling in many ecosystems over the past sixty years. Leaves of all plants have the same basic functions and all utilise the same suite of nutrients for the production of organic matter. Nutrient concentrations in individual tissues are more likely to reflect variation in soil fertility and so are useful when comparing the nutrient-status of different species and sites. ULRICH and HILLS (1967) stated that the concentration of a nutrient within a plant is an integrated value of all the factors that have influenced the nutrient concentration up to the time when the plant sample is taken. The nutrient concentration is dependent on two factors:

1. Nutrients accumulated up to the time of sample collection; and
2. Plant growth up to the time of sample collection.

SMITH and LONERAGAN (1997) regard the relationship between nutrient concentration and yield of a plant or plant part as forming the basis of most schemes for using plant analysis to assess plant nutrient status. Some common forms of relationships that have been observed are shown in Figure 2.7. The most common form (Figure 2.7a) comprises three parts: an ascending portion where yield increases with increasing nutrient concentration, a plateau where yield is not limited by nutrient concentration, and a descending portion in which yield declines with further increase in nutrient concentration. The complete form is dependent on the nutrient being studied, its initial level in the medium, the range of treatment levels applied, the plant part sampled, the time of sampling and the sampling procedure adopted. Variations in relationship are illustrated below (Figure 2.7 a, b, c).

It has been reported that in the youngest leaves of tomato, the yield is not affected by nutrient concentration, but sampling of older leaves reveals the common relationship with a more extended plateau (Figure 2.7b) (MARSCHNER, 1999). The sigmoidal relationship in the ascending portion of the curve in Figure 2.7c has been recorded for young plants growing in very deficient soils. The relationship shown in Figure 2.7d, in which initial increases in yield are associated with decreases in nutrient concentration was first noted in studies of copper nutrition and is referred to as the 'Piper-Steenbjerg effect' or C-shaped curvature and has been reported for a number of elements (SMITH and LONERAGAN, 1997).

Figure 2.7 Relationships between yield and nutrient concentration in plant parts



(SMITH and LONERAGAN, 1997)

2.5.2 Critical nutrient concentrations - A concept

According to ULRICH (1952), to diagnose nutrient deficiencies, standards must be developed to distinguish between plants with an adequate or a deficient supply of nutrients. These standards are generally referred to as 'critical nutrient concentrations', and are defined by three formal definitions:

- The nutrient concentration that is just deficient for maximum growth;
- The nutrient concentration that is adequate for maximum growth; and
- The nutrient concentration separating the zone of deficiency from the zone of adequacy.

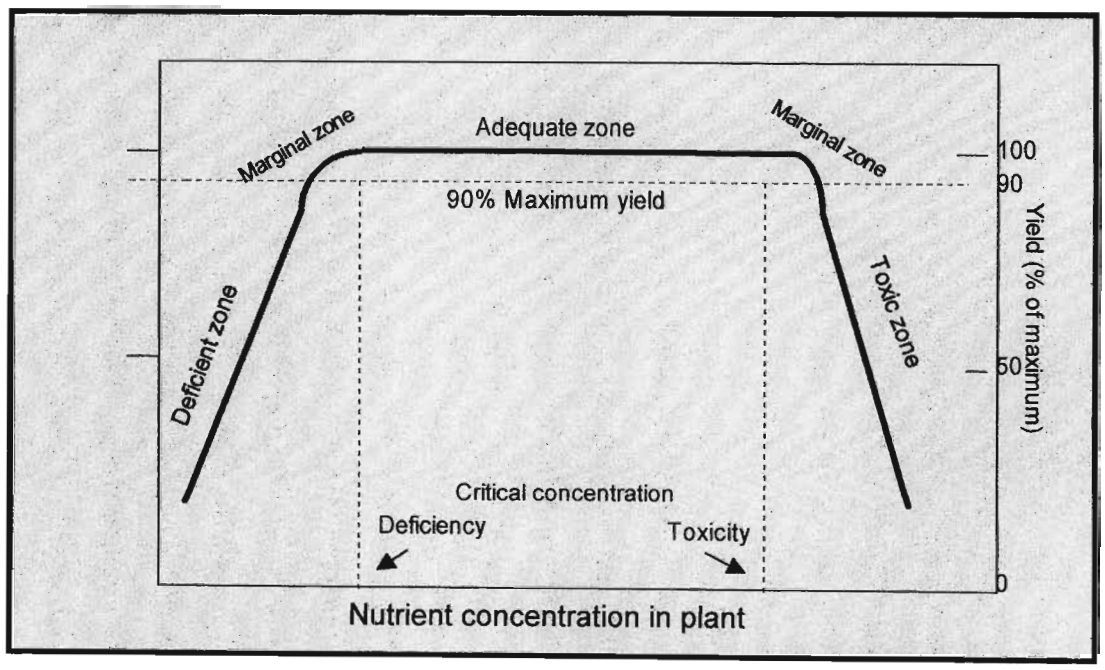
ULRICH (1952) proposed three definitions as the critical concentration is not a single value but a narrow range of nutrient concentrations, above which the plant is adequately supplied with nutrients and below which the plant is deficient. The view of the critical concentration being a range rather than a single value is often overlooked when critical concentrations are reported or used to interpret plant analyses. SMITH and LONERAGAN (1997) suggested that estimates of error values must be included in order to highlight that a critical concentration is a range. The fitting of non-linear regression models (step-wise regression) also permits the objective derivation of critical nutrient concentrations and their associated errors.

2.5.3 Derivation of critical nutrient concentrations

To derive a critical nutrient concentration for diagnosis requires that a well defined curve be established between nutrient concentration in a plant part and the current nutrient status of the plant. The standard measure of the plant's current nutrient status is the response of its shoot dry matter yield to nutrient supply and is expressed relative to the maximum shoot yield. Nutrient concentrations are generally expressed as the ratio of the total nutrient concentration to dry matter (DM) of a plant part - macro-nutrients as % DM and micro-nutrients as mg/kg DM (SMITH and LONERAGAN, 1997).

The critical concentration for deficiency diagnosis can be obtained from water culture, or field experiments in which increasing levels of nutrients are supplied to a deficient growing culture medium. An accurate measure of maximum yield is important. Following harvest of the appropriate plant part and analysis of its nutrient content, a relationship between nutrient concentration and shoot yield can be derived (Figure 2.8) (SMITH and LONERAGAN, 1997).

Figure 2.8 Concentrations for diagnosing nutrient deficiency and toxicity in plants



(SMITH and LONERAGAN, 1997)

An appropriate yield is then selected (90% of maximum yield) and the nutrient concentration in the plant part selected at this yield is accepted as the critical nutrient concentration. A similar approach is used for defining the critical concentration for nutrient toxicity and this can be more correctly considered a threshold of toxicity rather than a critical value (SMITH and LONERAGAN, 1997).

A number of researchers favour the use of a critical concentration range rather than a critical nutrient concentration. Critical concentrations are determined from the relationship between the yield and the nutrient concentration in a plant tissue (SMITH, 1962, DOW and ROBERTS, 1982). Generally, the most useful critical values are those that have been established for leaves of a specified development stage or age. The two most important criteria in choosing an organ for assay are the sensitivity of the response of the internal nutrient concentration to changes in the external nutrient supply and its stability towards factors other than the supply of the nutrient in question (DELL, 1996).

Leaves may be sclerophyllous as an adaptation to low availability of water or nutrient availability. MEDINA (1983) suggests that sclerophylly may be characterised by low concentrations of nitrogen and phosphorus and a high ratio of nitrogen to calcium. NEGI and SHARMA (1996) indicate that eucalypts grown in fertile soil in India have shown a lower degree of sclerophylly than those grown in infertile soil. This clearly indicates that eucalypts have a capacity to develop a different strain or **'ideotype'** according to the nutrient status of the soil. As sclerophylly decreases, there are associated decreases in leaf longevity, the residence time of nutrients in leaves and carbon gain. As the degree of sclerophylly increases so too does the longevity and residence time of nutrients. Thus, there should be an increase in nutrient use efficiency.

Foliar nutrient concentrations are sensitive to site differences and can play an important role in the identification of nutritional deficiencies and imbalances. Used in conjunction with fertiliser trials, this is a powerful means for maximising productivity through nutritional amendment. While much of the development of foliar analysis in agricultural crops has been aimed at deriving critical concentrations for individual nutrients, there has been a greater emphasis on identifying ideal ratios of nutrients in eucalypts (CROMER *et al.*, 1981, SCHÖNAU,1981, SCHÖNAU and HERBERT, 1983).

A number of workers have identified foliar nitrogen : phosphorus ratios as being particularly sensitive to additions of fertiliser and optimum values have been suggested for several commonly grown plantation eucalypts. CROMER *et al.* (1981) suggested an optimum nitrogen : phosphorus ratio of 15 for *E. globulus* and *E. sieberi* LAS Johnson. Analysis of *E. grandis* trials in South Africa led by Schönau and Herbert set the optimum at 13 (SCHÖNAU and HERBERT, 1989).

A second and often complementary approach to foliar analysis is through regression techniques, whereby foliar nutrient concentrations and ratios are compared alone or in combination with some index of tree growth. Using this approach LAMB (1977) explained 72% of the variation in height growth at age 15 months in *E. deglupta* Blume by foliar nitrogen and phosphorus concentrations alone. By including potassium concentrations and ratios of nitrogen, phosphorus and potassium JUDD *et al.* (1996) accounted for up to 74% of the variation in diameter growth for three *E. globulus* trials at age two-years. While regression techniques may be useful at an early age (up to two years), particularly for monitoring responses to fertiliser applied at establishment, their value diminishes thereafter. As trees mature and nutrient use and water use increase, competition limits growth. If one or more resources are scarce, growth may be affected at an early age. CROMER *et al.* (1981) found no relationship between concentrations of foliar nutrients and growth for a series of trials of a number of eucalypt species only four years after establishment.

OLSEN and BELL (1990) indicate that there have been few reports of positive relationships between growth and nutrient status in eucalypts, as the trees appear to have the capacity to utilise readily available nutrients to support rapid new growth, sometimes leading to a dilution of the tissue concentrations. CROMER (1996) discovered that the average concentration in foliage of two-year-old *E. globulus* was significantly **lower** in fertilised plots than in unfertilised plots, despite a foliage biomass almost six times greater in fertilised plots. As with most species, nutrient concentrations in eucalypt foliage also vary seasonally, with leaf ontogeny, with leaf age and with position in the crown (LAMB, 1976, BELL and WARD, 1984, LEUNING *et al.*, 1991). In a study of *E. diversicolor,* GROVE (1990) reported that twigs were a better indicator than leaf tissue when diagnosing deficiencies and predicting requirements for nitrogen and phosphorus. Analysis of bark may be preferable to leaf analysis for detecting deficiencies, and xylem sap analysis has also been suggested as a method of detecting nutrient deficiencies in trees (CROMER, 1996).

The three youngest mature leaf blades and undeveloped leaf blades are the most sensitive indicators of nitrogen status in eucalypt seedlings, whereas stems and petioles are better indicators for phosphorus (OLSEN and BELL, 1990).

Diagnostic and Recommendation Integrated System (DRIS) indices are a refinement of the nutrient ratio approach (SUMNER, 1977). DRIS indices calculated from the ratios of elements in fully expanded leaves from the upper crown of *E. saligna* in Western Australia predicted and

ranked nutrient deficiencies, whereas those calculated from other tissues did not (WARD *et al.*, 1985). DRIS indices calculated for *E. nitens* and *E. globulus* plantations in south-eastern Australia indicated substantial requirements for both nitrogen and phosphorus on ex-forest sites, and calcium on old agricultural sites (CROMER, 1996).

Using excised tree roots as an indicator for demand of phosphate, HARRISON and HELLIWELL (1979) showed that a measure of influx of ^{32}P-labelled phosphate solution into roots correlated well with tree growth and, inversely with phosphate supply to the tree. DIGHTON *et al.* (1993) used a modification of this bioassay technique to determine nitrogen, phosphorus and potassium demands by *E. grandis* seedlings in a greenhouse. Results indicated that the root bioassay was a more sensitive nutrient limitation than foliar analysis. The method was also used to assess nutrient response in a field experiment with *E. grandis* in South Africa and again showed that the bioassay was capable of indicating potential response of trees to fertiliser addition (JONES and DIGHTON, 1993).

Foliar samples taken when nutrient concentrations are relatively stable, and when trees are considered to be under maximum stress, are the most reliable for diagnostic purposes (WILL, 1985). Studies of the seasonal changes in foliar nutrient concentrations in young eucalypt stands showed that appreciable seasonal fluctuations can be expected for all nutrients. Data collected over a 25 month period suggested that foliar concentrations of nitrogen, phosphorus, calcium, copper and manganese in *E. saligna* are close to, or at, the minimum in midsummer (late February to March) and this may be an appropriate time to sample eucalypts in the Southern Hemisphere.

Not all nutrients follow the same pattern. Manganese concentrations appear to be lowest in spring (September - October), whilst the concentration of potassium may be lowest in about midwinter (KNIGHT and NICHOLAS, 1996). Thus, a single sampling time for overall evaluation of nutrient status may not be appropriate (BELL and WARD, 1984).

SCHÖNAU (1983) suggests that foliar analysis has been used mainly to determine the effectiveness of fertilisers and to examine the nutrient balances within the tree, rather than to identify critical values of nutrient concentrations in the leaves. Since optimally sited eucalypts grow virtually the whole year round, there is no dormant season. Consequently, sampling is at the height of the growing season (summer) when differences between nutrients are most pronounced.

SCHÖNAU (1981) examined the monthly variation in foliar nutrient concentrations of *E. grandis* between 12 and 36 months of age, over three sites. Concentration differences were most apparent between sites in summer and tended to decrease in winter. Foliar concentrations of nitrogen, phosphorus, sulphur and copper generally decreased with age, whilst those for potassium, calcium, magnesium, zinc and manganese were constant, and the concentration of iron tended to increase. Concentrations of nitrogen, calcium, sulphur, zinc and iron varied with rainfall, and concentrations of phosphorus and copper varied with temperature.

The effects ($P < 0.05$) of fertilising with nitrogen, phosphorus, potassium and dolomitic lime ($CaCO_3$) on the nutrient concentrations in the leaves of *E. grandis* has been studied by SCHÖNAU (1981), SCHÖNAU and HERBERT (1983) and HERBERT (1990). Liming increased foliar magnesium and zinc, but decreased manganese, potassium and calcium. Super-phosphate increased foliar phosphorus except where there were high levels of natural phosphorus and low zinc in the soil. Limestone-ammonium-nitrate (LAN) increased the levels of foliar nitrogen in soils with low contents of organic matter and decreased phosphorus where soil phosphorus was low. It was clear that added phosphorus and nitrogen interacted in their effects on foliar nitrogen and phosphorus, the nature of these interactions depending on the nitrogen and phosphorus status of the soil. The addition of potassium consistently increased foliar potassium, but decreased calcium where the exchangeable calcium in the soil was low. The concentrations of foliar nutrients confirmed the known shortages of nitrogen and phosphorus, and identified deficiencies of magnesium, zinc and sulphur for most sites (HERBERT, 1996).

In general, changes in nutrient concentration following the application of fertilisers are small and indicate that acute nutrient deficiencies are not typical (SCHÖNAU and HERBERT, 1983). The nitrogen : phosphorus ratio in foliage is a clear indicator of requirements of nitrogen and phosphorus fertilisers. Responses to nitrogen in fertiliser are expected where the ratio is low, and responses to phosphorus in fertiliser are expected where the ratio is high. The greater the deviation of this ratio from the optimum of 18, the more marked the growth response to the predicted fertiliser. Although the ratios of nitrogen : potassium, nitrogen : sulphur , phosphorus : potassium and calcium : magnesium may be useful, responses to fertilisers based on these ratios are complex and require multiple comparisons between fertilisers and ratios of foliar nutrients (HERBERT, 1996).

As ratios of foliar nutrients have proven to be the most meaningful in examining nutrient status, a more comprehensive approach using DRIS was investigated by HERBERT (1988) on the

Zululand sands which are low in soil organic matter. Calcium, copper and zinc were more growth limiting than nitrogen. This helped explain the poor response to LAN applications. The optimum foliar nutrient concentrations and some of their ratios are shown in Table 2.23. Although these figures are given for *E. grandis,* there is evidence that they may be utilised in assessing the nutrient status of other fast growing eucalypts (SCHÖNAU,1981).

HERBERT (1996) reported that increased nutrient uptake following fertiliser application does not necessarily indicate better growth, nor does a decrease in nutrient concentration result in poorer growth. The best growth is achieved when there is an overall balance of nutrients, i.e. where nutrient ratios all approach their optima. However, some ratios are more important than others and if the ratios of nitrogen : phosphorus, nitrogen : potassium, nitrogen : sulphur, phosphorus : potassium and calcium : magnesium are close to their optima, it is unlikely that growth rate can be increased through fertilising.

HERBERT (1991) also studied the relationships between climatic and edaphic factors and the concentrations of foliar nutrients of *E. grandis* on seven sites. Very good correlations were found between the properties of the topsoil (pH, exchangeable acidity, organic carbon, exchangeable calcium, magnesium, potassium and available phosphorus) and all concentrations of foliar nutrients, especially nitrogen and phosphorus followed by copper, calcium, sodium, iron and aluminium. The most important properties of the top soil affecting foliar nutrients were the amounts of exchangeable potassium, extractable phosphorus, pH and exchangeable calcium. Multiple regressions explained 78 to 97% of the variation in concentrations of nutrients.

Table 2.23 Optimum foliar nutrient concentrations and some of their ratios for *E. grandis* in South Africa

Foliar nutrient or ratio		Range	
	Optimum	Minimum	Maximum
N (%)	2.80	1.25	3.35
P (%)	0.15	0.10	0.35
K (%)	0.75	0.36	1.19
Ca (%)	>1.0	0.56	1.82
Mg (%)	0.35	0.21	0.62
S (%)	0.20	0.10	0.29
Na (%)	0.32	0.11	0.46
Mn (mg/kg)	600.00	129.00	6005.00
Fe (mg/kg)	110.00	52.00	1021.00
Al (mg/kg)	160.00	29.00	353.00
Zn (mg/kg)	18.00	8.00	32.00
Cu (mg/kg)	12.00	2.00	26.00
B (mg/kg)	32.00	15.00	47.00
N:P	18.00	3.00	28.60
N:K	3.50	1.00	5.40
N:S	14.00	3.90	17.50
P:K	0.20	0.09	0.62
Ca:Mg	>3.3	1.24	7.28

(HERBERT, 1996)

Very few critical values have been set for eucalypts. Until critical nutrient concentrations have been defined for all the essential nutrients and for all the commercial species of eucalypts, standard nutrient concentration ranges provide an acceptable alternative. These standards divide the yield response curve into broad regions:

- Deficient trees;
- Marginally deficient trees; and
- Trees with adequate nutrients.

An example of ranges of standard nutrient concentrations for *E. maculata* seedlings is shown in Table 2.24. Whilst these values can be obtained experimentally, they can also be obtained from the analysis of high and low yielding trees in the field (DELL, 1996).

Table 2.24 Plant analysis guide to deficient and non-deficient *E. maculata* seedlings.

Plant parts: SA - shoot apex; EL- expanding leaves; YFEL - youngest fully expanded leaves. Deficient - plants with severe symptoms; Marginal - the state of the plants when first symptoms appear; Adequate - plants without symptoms, supplied with complete fertiliser

Nutrient	Plant part	Deficient	Marginal	Adequate
			mg/g dry weight	
N	YFEL	10-12	-	17-26
P	YFEL	0.4-0.5	0.8	1.0-2.6
K	YFEL	4	5-7	10-17
S	EL	1.2-1.3	1.4-1.6	1.8-4.2
Ca	YFEL	1.5-2.0	2.1	2.9-4.0
Mg	YFEL	0.3	0.4-0.6	0.9-2.4
			µg/g dry weight	
Mn	YFEL	12-15	20	22-32
Cu	SA	0.5-1.5	2	6-12
Zn	SA	4-8	9	12-54
Fe	YFEL	15	20-30	39-50

(DELL and ROBINSON, 1993)

SMITH and LONERAGAN (1997) suggest three main procedures in establishing nutrient concentration standards for the diagnosis of the nutrient status of plants. The first uses nutrient addition experiments to provide response curves to define those plant nutrient concentrations which limit growth through deficiency or toxicity. By defining the nutrient concentrations for both deficiency and toxicity, the procedure also defines the sufficiency range. The second procedure uses nutrient concentrations in samples from surveys of crops in the field. The third procedure, 'Vector Analysis' has recently been suggested for diagnosing nutrient deficiencies in forest trees. This is not a diagnostic procedure but is simply an aid to the interpretation of responses to fertiliser or other treatments. It should not be confused with the statistical procedure of vector analysis. It is much simpler and involves graphical representation of the relative changes in dry weight and nutrient concentrations and contents of leaves or shoots in response to nutrient treatments.

2.6 Factors affecting nutrient activity and uptake

2.6.1 The importance of pH control

According to BUGBEE (1996), plants grow equally well between pH 4 and pH 7, if nutrients do not become limiting. This is because the direct effects of pH on root growth are small, and the real issue is reduced nutrient availability at high and low pH. The recommended pH for hydroponic culture is between 5.5 to 5.8 as overall availability of nutrients is optimised at a slightly acid pH. The availability of manganese, copper, zinc and especially iron is reduced at higher pH, and there is a small decrease in availability of phosphorus, potassium, calcium and magnesium at lower pH. Reduced availability means reduced nutrient uptake, but not necessarily nutrient deficiency. COOPER (1996) does not fully concur with this pH range and suggests that for most crops, the pH should be 6.0 to 6.5.

MATSUI (1995) experimented on the hydroponic culture of coniferous seedlings using two and three-year-old Japanese cedar *(Cryptomeria japonica)* and Japanese cypress *(Chamaecyparis obtusa)*, to test seedling tolerance to acidity. Nine major nutrients (Na^+, NH_4^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, NO_3^-, PO_4^{3-} and SO_4^{2-}) were supplied in the water solution and growth of trees and foliar damage was observed for two years. The culture solution was changed every 20 days, or more frequently during periods of rapid growth. Nutrient uptake by the seedlings was estimated from daily changes in nutrient concentrations in the culture solution. The effect of acidity on the culture solution was also tested. The pH of the 'normal' solution used was over 7.0 (7.11-7.33) when fresh, falling to 3.20-4.32 during use. Some 50% of the Japanese cedar seedlings and 30% of Japanese cypress seedlings lived for two years under the normal experimental conditions, but seedlings raised under strong acid conditions were dead within three months.

As hydroponic systems are poorly buffered it is difficult to keep the pH between 4 and 7 without automatic pH control. Phosphorus (H_2PO_4 to HPO_4) in solution buffers pH, but if phosphorus is maintained at levels that are adequate to stabilise pH (1-10 mM), it becomes toxic to plants. Plants actively absorb phosphorus from solution so a circulating solution, with approximately 0.05 mM P has much less buffering capacity than the fresh refill solution that is added to replace transpiration losses. A fresh refill solution that is buffered by phosphorus has its maximum buffering capacity at pH 7.2. This point is called the pKa of the buffer and it is the point at which half of the phosphorus is in the H_2PO_4 form and half is in the HPO_4 form. In other words, the phosphate ion absorbs and desorbs hydrogen ions to stabilise the pH. Unfortunately, phosphorus is quickly removed from solution and needs to be replenished up to three times a day (BUGBEE, 1996).

2.6.2 Why pH varies

The ratio in uptake of anions (negatively charged nutrients) and cations (positively charged nutrients) by plants may cause substantial shifts in pH. In general an excess of cations over anions leads to a decrease in pH, whereas an excess of anion over cation uptake leads to an increase in pH. As nitrogen may be supplied either as a cation (ammonium - NH_4^+) or an anion (nitrate - NO_3^-), the ratio of these two forms of nitrogen in the solution can have large effects on both the rate and direction of pH changes with time. This shift in pH can be surprisingly fast (ERREBHI and WILCOX, 1990).

Daylight photosynthesis produces hydrogen ions which can cause the nutrient acidity to increase (lowering the pH). At dusk photosynthesis stops and plants increase their respiration rate. This, coupled with the respiration of micro-organisms and the decomposition of organic matter, uses up the hydrogen ions and the acidity of the solution decreases (pH rises). In low light, plants take up more potassium and phosphorus from the nutrient solution and acidity increases (pH drops). In intense light, plants take up more nitrogen from the nutrient solution and the acidity decreases (pH rises) (ANON, 1996).

2.6.3 The effect of pH fluctuation on the precipitation of certain nutrients

BUGBEE (1996) states that plants exhibit anomalies through leaf symptoms (e.g. iron deficiency) when it's too late. Iron is one essential plant nutrient whose solubility is affected by pH, which is why it is added in a chelated form. At pH 7.0, less than 50% of the iron is available to plants. At pH 8.0, no iron is left in solution due to iron hydroxide precipitation ($Fe(OH)_3^-$). As long as the pH is kept below 6.5, over 90% of iron is available to plants.

The pH of nutrient solutions also affects the solubility of calcium and phosphorus. Due to calcium phosphate precipitation ($Ca_3(PO4)_2$), the availability of calcium and phosphorus decreases at pH values above 6.5. All other nutrients stay in solution and do not precipitate over a wide pH range. Poor water quality can exacerbate precipitation reactions. Generally, in the pH range 4.0 to 6.0, all nutrients are available to plants. Precipitation reduces iron, calcium and phosphorus at pH 6.0 and over (ANON, 1996).

2.6.4 Adjusting pH

Nitrogen is the essential inorganic nutrient required in the largest quantity by plants. Most plants are able to absorb either nitrate (NO_3^-) or ammonium (NH_4^+) or both. Ammonium as the sole source of nitrogen or in excess is deleterious to the growth of many plant species. Some

plants yield better when supplied with a mixture of ammonium and nitrate compared to nitrate alone. A combination of ammonium and nitrate can be used to buffer against changes in pH (BUGBEE, 1996).

Plants grown in nutrient solution containing only nitrate as the sole nitrogen source tend to increase solution pH, hence the need to add acid. But when approximately 10-20% of the total nitrogen is supplied as ammonium, the nutrient solution pH is stabilised at pH 5.5. Ammonium concentration needs to be monitored as it has been recently shown that micro-organisms growing on plant root surfaces can convert the ammonium to nitrate. Since hand-held ion-selective electrodes for measuring both ammonium and nitrate are now available, it is possible to accurately monitor and maintain a predetermined nitrate : ammonium ratio throughout the life of the crop (ANON, 1996).

Phosphorus is required in large amounts by plants. Interestingly, there are two forms of fertilisers containing both potassium and phosphorus: 1). KH_2PO_4 mono-potassium phosphate (MKP) and 2). K_2HPO_4 di-potassium phosphate (DKP). Equal quantities of both can be used to maintain the pH at 7.0.

According to COOPER (1996), suitable acids to use for pH adjustment include phosphoric acid (H_3PO_4) and nitric acid (HNO_3). A suitable base is potassium hydroxide (KOH). If there are high calcium levels in the water, the use of nitric acid is advised, because more phosphoric acid than nitric acid would have to be used. If calcium is present in the water supply, the amount of acid required will be determined by the concentration of calcium bicarbonate ($Ca(HCO_3)_2$) present, because both phosphoric and nitric acid react with calcium bicarbonates as follows:

$$Ca(HCO_3)_2 + 2(HNO_3) = Ca(NO_3)_2 + 2CO_2 + 2H_20$$

$$Ca(HCO_3)_2 + 2(H_3PO_4) = Ca(H_2PO_4)_2 + 2CO_2 + 2H_20.$$

With either acid, both carbon dioxide (CO_2) and water (H_20) are produced, but with nitric acid, soluble calcium nitrate ($Ca(NO_3)_2$) is also produced, whereas with phosphoric acid, insoluble calcium phosphate ($Ca(H_2PO_4)_2$) is produced. Insoluble calcium phosphate is a white precipitate and has no nutritional value because of its insolubility, but there is a loss of some phosphorus. This reaction only occurs to a major extent when there are high calcium concentrations in the water. It is of some concern is that nitric acid is very corrosive whilst phosphoric acid is not.

Another method of pH stabilisation is the use of ion-exchange and chelating resins. Generally, these resins are small beads which have nutrients absorbed or chelated onto them. The nutrient solution circulates through the beads or the beads are suspended in the nutrient tank. As plants absorb nutrients, more nutrients are released by the resins. The aim is to achieve controlled release of nutrients into the solution in an attempt to mimic the way the soil releases nutrients. Ideally, such release can adequately supply the growing plant's nutritional requirements and maintain pH stability (ANON, 1996).

2.7 Hydroponic systems

2.7.1 Introduction to soilless culture and its requirements

HARRIS (1987) states that "hydroponics is the science of growing plants in a medium, other than soil, using mixtures of essential plant nutrient elements dissolved in water". The term 'hydroponics', derived from two Greek words 'hydro' (water) and 'ponos' (labour), was coined by Dr. WF Gericke of California, who grew tomatoes in plant nutrient solutions with spectacular results.

BENTON-JONES (1999) describes the most 'ideal' hydroponic system as having to fulfil the following requirements:

- Maintains a continuous supply of water under all environmental conditions;
- Provides all essential elements at specific concentrations and proportions at all times; and
- Controls root growth.

Hydroponics is a very young science that has been used on a commercial basis for only 50 years. The large increases in yields under hydroponic culture over that of soil are due to several factors. In many cases, agricultural lands lack the nutrients or have a poor soil structure from continual mechanised operations. The presence of ever more chemically resistant pathogens and insects due to the abuse of chemical sprays in monocultural operations, has also greatly reduced overall production. The main advantages of hydroponics over soil culture are more efficient nutrient regulation, suitability to regions of the world having non-arable land, efficient use of water and fertilisers, ease and low cost of sterilisation of medium, and higher-density planting leading to increased yields per unit area (Table 2.25) (RESH, 1998).

Table 2.25 Comparison of soilless culture versus soil culture

Cultural practice	Soil	Soilless
Sterilisation of growing medium	Steam, chemical fumigants; labour intensive; time required min 2-3 weeks	Steam, chemical fumigants with some systems; short time required to sterilise
Plant nutrition	Highly variable, localised deficiencies, often unavailable to plants due to poor structure or pH, unstable conditions, difficult to sample, test and adjust	Completely controlled, relatively stable, homogenous to all plants, readily available in sufficient quantities, good control of pH, easily tested, sampled and adjusted
Plant spacing	Limited by soil nutrition and available light	Limited only by available light; closer spacing possible; increased number of plants per unit area
Weed control & cultivation	Weeds present, cultivate regularly	No weeds, no cultivation. Coarse media voids in gravel provide soil air required by plant
Diseases and soil inhabitants	Many soil-borne diseases, nematodes, insects and animals that attack crops; crop rotation to overcome build-up of infestation. Certain level of buffering against rapid pathogenic attack	No diseases, insects, animals in medium; no need for crop rotation. Introduced soil-borne pathogens and insects may spread quickly to all beds on the same nutrient tank of a closed system
Water	Plants subjected to water stress due to poor soil-water relations, soil structure and low water-holding capacity. Saline waters cannot be used. Inefficient use of water; much lost as deep percolation past root zone; evaporation from the soil surface	No water stress. Complete automation by moisture sensing devices and feed back control mechanism; reduces labour costs, can use relatively high saline waters, no loss to percolation beyond root zone or surface evaporation; water loss = transpirational loss
Fertilisers	Broadcast large quantities over soil, non-uniform distribution to plants, large amount leached past root zone (50-80%) inefficient use	Small quantities utilised, distributed uniformly to all plants, no leaching beyond root zone, efficient use
Sanitation	Organic wastes used as nutrient source on edible portions of plant cause many human diseases	No biological agents added to nutrients; no human disease organisms present on plant
Transplanting	Need to prepare soil, uprooting plants leading to transplant shock. Difficult to control edaphic factors; disease organisms may kill or retard transplants	No preparation of medium required prior to planting; transplant shock minimised, faster 'take' and subsequent growth. Medium temperature can be adjusted by flooding with nutrient solution. No diseases present
Plant Maturity	Limited ability to manipulate maturation	With adequate light conditions, plant can mature faster
Permanence of medium	Soil in greenhouse must be changed every several years as fertility and structure break down. Under field conditions, must fallow	No need to change medium in gravel, sand or water cultures; no need to fallow. Sawdust, peat, vermiculite last several years between changes
Yields	Greenhouse tomatoes 7-12 kg/year/plant	11-16 kg/year/plant
Labour requirement	Highest of costs to agriculture. Constant cultivation of crops required. Labour can be less educated	Menial nature of cultivation eliminated. Trained staff must direct the growing operation. Knowledge of plant biology and principles of nutrition essential
Revitalising of stock plants	Not always possible to revitalise valuable stock plant *in-situ* once stress is noted	Best method of revitalising poor plants. Achieved through balanced nutrient levels available in hydroponic system
High initial construction costs	Cost of establishment of crop lower	Development costs of commercial hydroponics system high
Attention to detail	Buffering action of soils reduces effect of poor cultural practices	Response of plant to good or poor nutrition rapid. Grower must observe crop every day

(HARRIS, 1987, BENTON-JONES, 1998, RESH, 1998, JENSEN, 1999)

2.7.2 Types of Hydroponics systems

JENSEN (1999) describes hydroponics as "a technology for growing plants in nutrient solutions with or without the use of an artificial medium to provide mechanical support". Liquid hydroponic systems have no supporting medium for plant roots whilst aggregate systems have a solid medium of support. Some publications persist in confining the definition of hydroponics to liquid systems only. This exclusion of aggregate hydroponics serves to blur statistical data and may lead to an underestimation of the extent of the technology and its economic implications.

Two types of aggregate (including sand, gravel, vermiculite, rockwool, peatmoss, coir, sawdust) culture have emerged; namely open systems and closed systems. In open systems, nutrient solution is supplied to the aggregate and any excess liquid drains to waste. In closed systems, the aggregate is moistened with the nutrient solution, and the draining liquid is collected and reused. A myriad of different hydroponic designs have been developed for a number of specific roles. The aim of this section is to give a generalised overview of the two distinct types of commercial hydroponic systems: i.e. recirculating and non-circulating, each of which has distinct applications (COOPER, 1996).

2.7.2.1 Recirculating systems (closed systems)

The basic concept behind the recirculating system is that a nutrient solution is collected and reused, either 'as is' for a period of time before being replenished with fresh water and nutrients, or continuously replenished by computer controlled equipment. These systems have the advantage of limiting excess fertiliser run off from nurseries and thus reducing environmental pollution, as well as optimising the use of fertilisers and water (A'BEAR, 1995).

2.7.2.1.1. Liquid (non aggregate) hydroponic systems

Liquid systems are closed systems in which the plant roots are directly exposed to the nutrient solution, with no other growing medium, and the solution is reused. There are several systems in this category including the **Nutrient film technique** (NFT) and **Deep flow hydroponics** (DFH) (JENSEN, 1999).

2.7.2.1.1.1. Nutrient film technique (NFT)

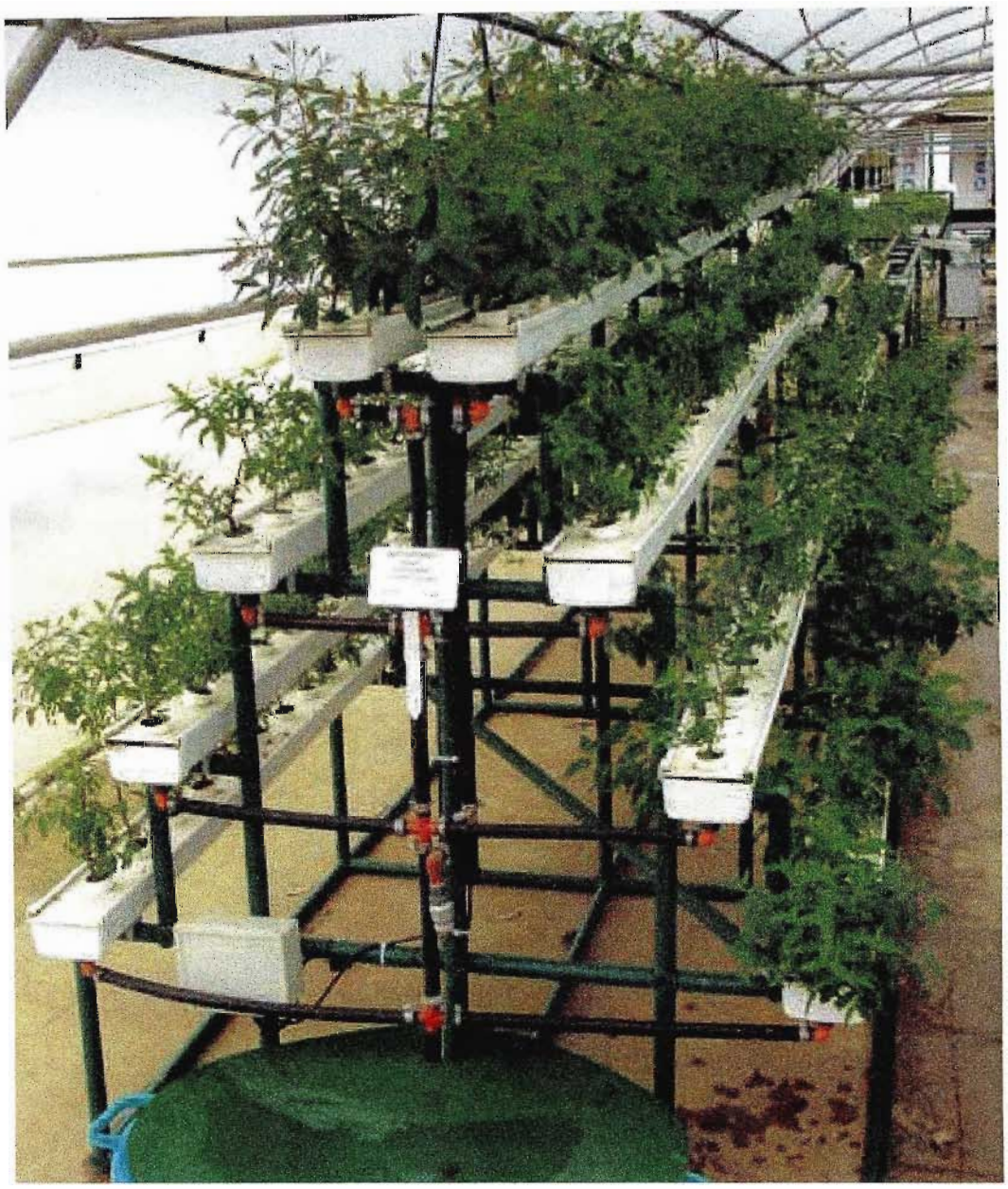
NFT is possibly the purest form of hydroponics in that plants are suspended with their roots coming into contact with a film of nutrient solution. The flow of water must be continuous and at a rate to prevent stagnation (A'BEAR, 1995). PAPADOPOULOS (2000) concurs with the

previous statement and notes that of all the soilless methods, water culture by definition, may be regarded as a true hydroponic system. NFT is a relatively new water-culture system based on the simple principle of circulating a shallow stream, or film, of nutrient solution over the roots of growing plants to provide an adequate supply of water, nutrients, and oxygen (Figure 2.9). The concept of a nutrient film is credited to AJ Cooper, who while at the Glasshouse Crops Research Institute in Littlehampton, England, recognised its value and called international attention to its commercial potential as early as 1973. Since then, NFT has undergone intensive testing by scientists and commercial growers in many countries, and is now considered a commercially viable form of water culture for several crops (COOPER, 1996, JENSEN, 1999).

According to PAPADOPOULOS (2000), the basic components of a typical NFT installation are:

- Parallel gullies, or troughs, in which to grow the plants laid on a 1-2% slope, on which the nutrient solution flows (Table 2.9);
- A catchment tank containing nutrient solution where fertilisers, water, and acid are added;
- A circulation pump that draws solution from the catchment tank and delivers it to the upper ends of the gullies;
- A catchment pipe to collect solution discharged from the gullies;
- Fertiliser and acid supply tanks to store concentrated fertiliser stock solutions and an acid solution; and
- Monitoring and control equipment to maintain nutrient concentrations (including total amount of salts), pH, and water level. An electrical conductivity (EC) controller and a pH controller are commonly used to regulate the operation of dosimetric pumps or solenoid valves. These pumps, or valves, control the transfer of fertilisers and acid to the catchment tank. A mechanical floating valve or a variety of electronic controls easily maintain a constant water level in the catchment tank.

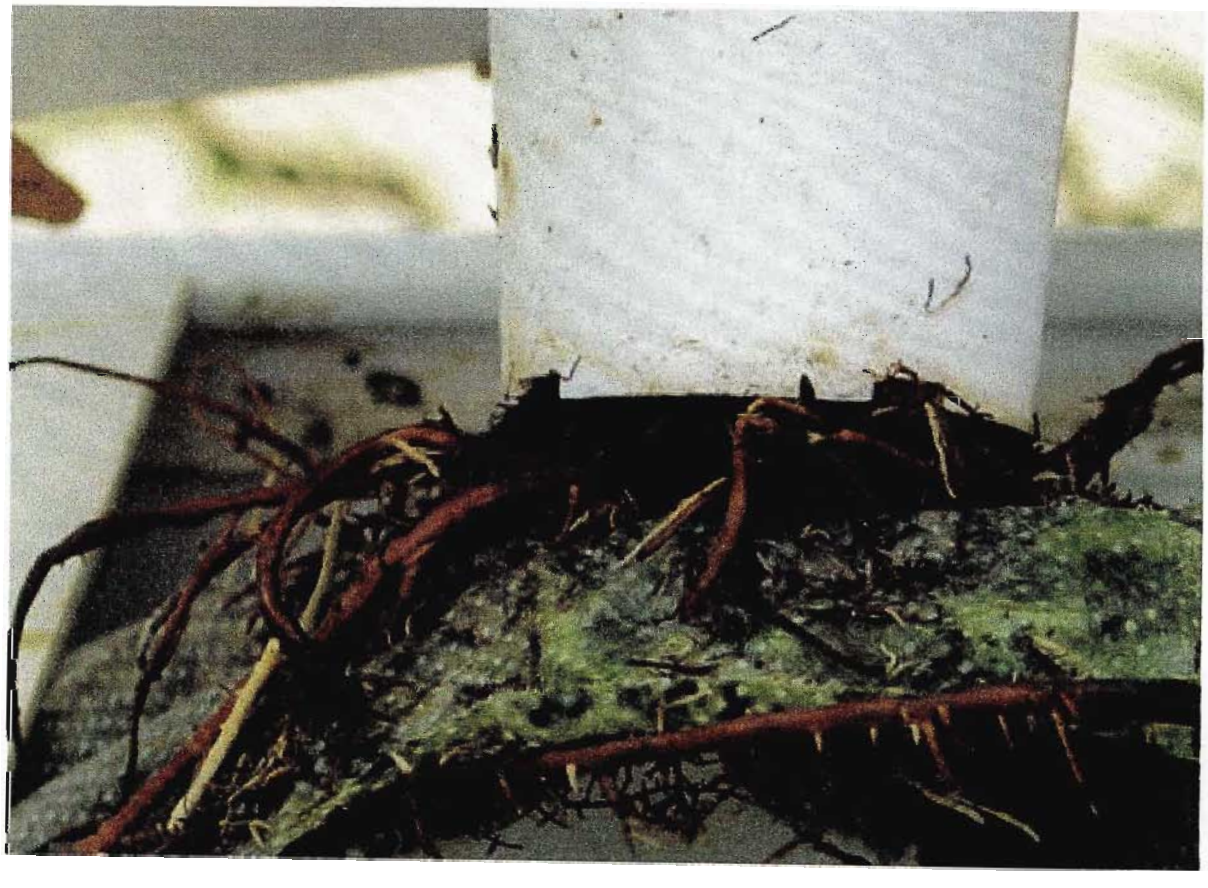
Figure 2.9 A commercial NFT system designed for the propagation of leafy vegetables. The unit has proven to be successful as a platform for the sustaining of hydroponic clonal eucalypt hedges



NFT has many advantages over other systems of crop production. It has been designed for simplicity, low cost and dependability. In particular, it gives absolute control of the root environment, it greatly simplifies watering and ensures a uniform nutrient supply across the whole crop. Root temperature can be raised easily whenever required merely by warming the nutrient solution, which can be circulated either continuously or intermittently to further conserve energy and to control the vegetative growth of young winter-grown plants (PAPADOPOULOS, 2000).

According to COOPER (1996), in conventional agriculture, if an abundance of water is provided there is inadequate air at the root surface. As the soil dries out, air penetrates. When there is an abundance of oxygen there is inadequate water. Thus, in conventional agriculture the balance between the supply of oxygen and water at the root is continually changing, and usually one or the other is limiting. In an NFT crop there is a permanent abundance of both air and water at the root, provided the nutrient film is maintained (Figure 2.10).

Figure 2.10 Root proliferation of a eucalypt hybrid clone in a NFT system. Capillary matting ensures an even distribution of nutrient solution. Note the development of white carbohydrate enriched adventitious roots



Other advantages include a rapid turnaround between successive crops, the potential for more efficient use of greenhouse space because of the possibility of plant mobility, and the potential for more efficient use of water. NFT's high degree of control over nutrition, water availability, and root environment (Figure 2.10) makes it the most sophisticated of all commercial plant-culture systems in practice today. Theoretically it offers the highest yield potential.

Many of the advantages of NFT are also offered by other soilless methods, notably rockwool. Much scepticism persists about the future of NFT, because it is generally perceived as a technique that requires a high level of technical skill.

Growers have some concern about the possibility that a recirculating nutrient solution may amplify and spread diseases in the system, resulting in disastrous crop losses. Unexplainable outbreaks of root death have repeatedly occurred, which have fuelled concern over potential spread of diseases in NFT cucumbers. The NFT method, the deep-culture technique (pioneered in Japan), and other closed-loop hydroponic methods are now being re-examined with renewed interest because of their potential for minimising fertiliser waste and environmental pollution (PAPADOPOULOS, 2000).

NFT was originally developed as a low-cost system using lightweight, disposable gullies and simple salinity and pH controllers. However, as the system became a commercial reality, it became increasingly automated, standardised, and sophisticated, which made the capital cost of the initial installation a major concern for growers contemplating its use. As a general recommendation, PVC and low and high-density polyethylene or polypropylene are acceptable, but not plasticised PVC (used in the manufacture of flexible hose) or butyl-rubber sheet lining (used for waterproofing reservoirs) as they may be phytotoxic. Plastic surfaces quickly lose their potential phytotoxicity when exposed to a nutrient solution.

To ensure good root aeration, an adequate rate of flow of at least 2 L/min is required and a depth of solution of no more than 1 cm, even when the root mat is well developed (PAPADOPOULOS, 2000). Although NFT at first relied on sloping soil surfaces, occasionally made of concrete, an increased interest is now evident in raised systems using rigid platforms, which support the gullies, and in adjustable stands (Figure 2.9). Such systems eliminate pockets of deeper solution resulting from poor soil levelling and allow for slope adjustment, even during cropping. Furthermore, a raised NFT system can be installed and operated in an old greenhouse, where grading the soil might be difficult or even impossible. Widely available fibre glass or plastic containers have been used as catchment tanks, but because they are usually small, their usefulness is limited to small NFT installations.

Various techniques have been developed to further increase oxygenation of the nutrient solution. Two separate return pipes arranged to enter the catchment tank at right angles to each other so that the nutrient solution streams converge well above the solution in the tank, will aid with agitation. A more deliberate attempt to increase mixing and aeration of the nutrient

solution in the catchment tank involves the direct return, under pressure, of some of the nutrient solution pumped by the main circulation pump.

Fertilisers and acid are normally added to the catchment tank in the form of concentrated stock solutions. The dosimetric pumps used to inject nutrients and acids into the catchment tank need to be chemically resistant, at least in those parts that come into contact with the relatively concentrated solutions (PAPADOPOULOS, 2000). Two pumps are required for fertiliser and one for acid; their size depends on the size of the operation, but an average capacity of 10 L/h is normal.

A salinity controller provides the best method for determining the salt concentration by measuring and controlling the electrical conductivity (EC) of the solution. This method uses the principle that the electricity conducted between two electrodes, immersed at a fixed distance (usually 1 cm) in a solution, is proportional to the total ionic (salt) concentration in that solution. The EC controller monitors and displays the conductivity of the nutrient solution and activates the metering (dosimetric) pumps when the measured conductivity falls below a pre-set value and only until the measured value regains the pre-set value.

Electrical conductivity is usually reported in either microSiemens per centimetre (µS/cm) or micromho per centimetre (µmho/cm). Other units and conventions are used occasionally to express EC, but the relationships between them are straightforward: for example, 1 milliSiemen (mS) = 1 millimho (mmho) = 1000 microSiemens (µS) = 1000 micromho (µmho) = 10 conductivity factor (CF) units; reference to centimetres is usually omitted, but implied. The cells (sensors) used in conductivity measurements are encased in plastic, which makes them sturdy, requiring only minimal maintenance (PAPADOPOULOS, 2000).

Two main types of conductivity cells are available: a dip cell suspended in the solution and suitable for small installations, and a flow-through type cell incorporated in the pipeline. In the latter case a sampling loop returns some of the main circulating pump's output solution directly back into the catchment tank after it has passed through the conductivity cell. The electrical conductivity of a solution increases by about 2% for every degree Celsius that the temperature increases. A conductivity controller with automatic temperature compensation is a standard option in most conductivity controllers (PAPADOPOULOS, 2000).

Where the water supply contains nutrients in excess of plant requirements, or where the fertilisers are not supplied in a ratio proportional to nutrient uptake by the crop, a build-up of

certain nutrients inevitably results. Nutrients that can accumulate over time include calcium (from hard water), sulphate (from fertilisers), sodium and chloride (from saline water) (PAPADOPOULOS, 2000). Under these conditions the background conductivity rises progressively, so proportionate increases in the EC setting of the salinity controller are needed to maintain an adequate nutrient supply. The nutrient solution must be discarded periodically and new solution added to the system. The frequency at which to renew the nutrient solution depends on the stage of crop growth and the season, both of which affect the rate of nutrient and water uptake by the crop.

2.7.2.1.1.2 Deep flow hydroponics

In 1976, a method of growing a number of lettuce and other leafy vegetables on a floating raft of expanded plastic was developed independently by Jensen in Arizona and Massantini in Italy. The system consists of horizontal, rectangular shaped tanks lined with plastic measuring 4 x 70 m x 0.3 m deep. The nutrient solution is monitored, replenished, recirculated and aerated (JENSEN, 1999).

2.7.2.1.2. Aggregate hydroponic systems

JENSEN (1999) states that in aggregate systems, a solid inert medium provides support for the plant. As in liquid systems, the nutrient solution is delivered directly to the roots. Aggregate systems may either be open or closed, depending on whether surplus amounts of the solution are to be recovered and reused.

In most open hydroponic systems, excess nutrient solution is recovered. However, the surplus nutrient is not recycled to the plants, but is disposed of in evaporation ponds or used in adjacent irrigation schemes. Because the nutrient solution is not recycled, such open systems are less sensitive to the composition of the medium used or the salinity of the water. In addition to a sand medium spread across a greenhouse floor, open systems may also use troughs, trenches, bags and slabs of horticultural grade rockwool (JENSEN, 1999).

2.7.2.1.2.1 NFT/Gravel culture

This is an adaptation of the NFT concept in that the channel has a layer of 6 to 9 mm diameter stone laid in it (Figure 2.11). This allows the channel to be widened and take on the proportions of a bed up to one metre wide. A nutrient solution is pumped through the gravel which acts as an anchor for the root system.

This system is by far the most popular choice for commercial growers for a number of reasons:

a. Channels are laid onto the prepared ground so that no construction tables are needed;
b. Channels can be constructed from plastic sheeting;
c. Stone is readily available in different sizes;
d. Shade structures are adequate and rains can flush out salt build-up in the system;
e. Relatively simple to maintain;
f. Perfectly suited to high value crops such as celery, lettuce, spring onions and herbs; and
g. Can survive a period of reduced or no water flow as stones hold a certain amount of moisture (A'BEAR, 1995, RESH, 1998).

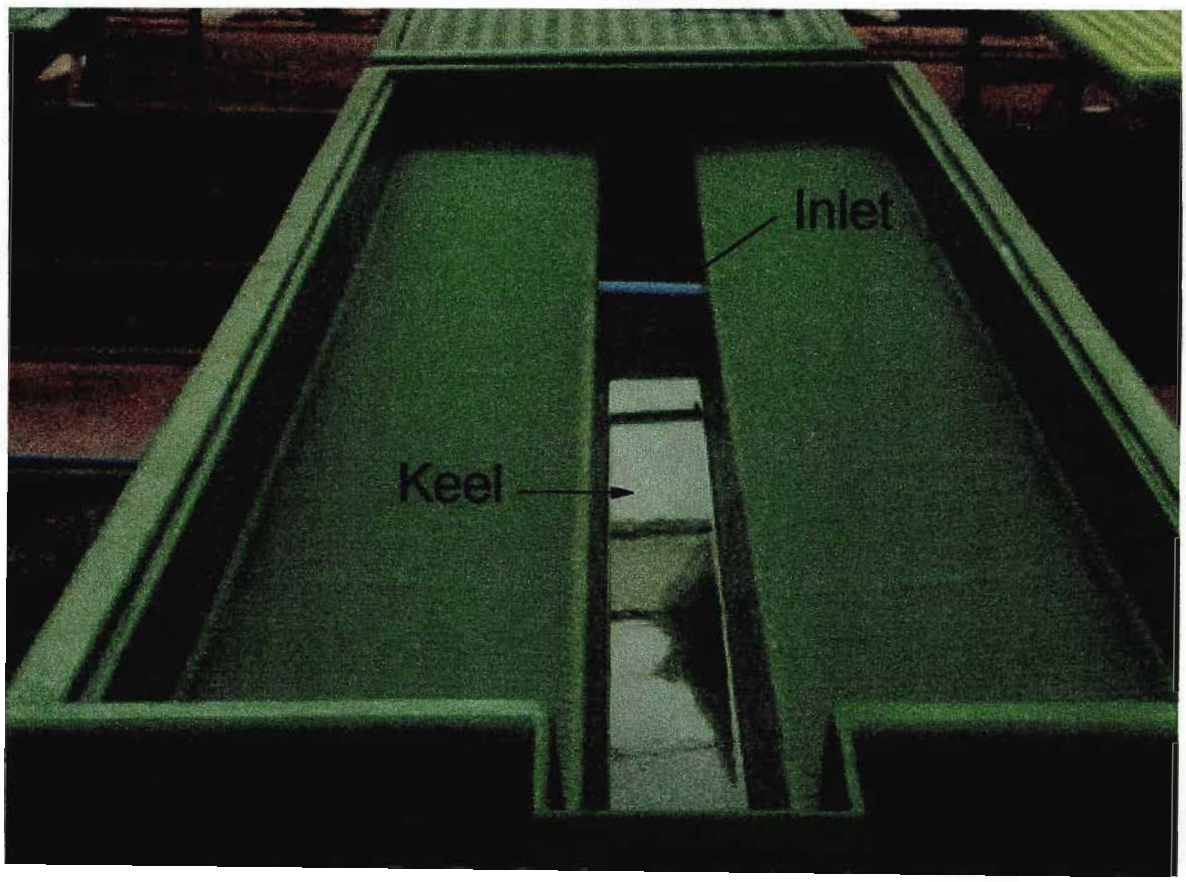
Figure 2.11 NFT/Gravel culture system developed at Mondi Forests to compare the growth response of *Eucalyptus* hybrid clones to different substrates. The system was further modified to include sand and perlite

2.7.2.1.3 Sub-irrigation or ebb and flow

RESH (1998) describes the ebb and flow techniques as a sub-irrigation method. Nutrient solution is pumped into a shallow bed to a depth of 2 to 3 cm for about 20 minutes and then allowed to drain back to the nutrient tanks once the pumps are shut off. A'BEAR (1995) notes that this is not a very popular commercial system due to the expense of construction.

Sub-irrigation is particularly suited to long term crops such as tomato, cucumber and peppers because of the larger volume available to the root systems as compared to that of NFT channels. An added advantage is that the larger, deeper trough draws air down the full depth of the bed each time the bed is flooded and drained (Figure 2.12).

Figure 2.12 Fibre glass ebb and flow system designed and manufactured for Mondi Forests. The units house superior eucalypt hybrid crosses for commercial planting



The basic requirements for a sub irrigation system are:

a. Troughs 1.0 to 1.5 m wide and 0.5 m deep, water tight, up to 30 m long (Figure 2.12);
b. Floor of the trough slopes to the centre down the length to facilitate drainage;
c. An inlet port for nutrient solution and for draining;
d. Coarse sand or gravel as alternative substrate;
e. Sump tank;
f. Header tank;
g. Recirculating pump for returning solution to header tank;
h. Solenoid valves for controlling the filling and draining of the tank; and
I. Perfectly level construction (A'BEAR, 1995).

2.7.2.1.3.1 Gravel culture as a sub-irrigation technique

Gravel culture was the most widely used hydroponic technique from the 1940s through the 1960s. Today, NFT rockwool and perlite cultures are more widely used as they have more consistent properties, are easier to sterilise between crops and are less laborious to handle, maintain and manage (Figure 2.11) (RESH, 1998).

A'BEAR (1995) and RESH (1998) regard gravel culture as the most widely used commercial method of hydroponics, and it is virtually synonymous with sub-irrigation. The nutrient solution is stored in an external reservoir from where it is pumped into the gravel through a pipe at the end of a waterproof bed and the solution percolates through the gravel. When the pump is switched off, the solution flows back into the reservoir for topping up and reuse. The word 'gravel' refers to the aggregate size, not geological origins of the stone, and the usual limits are 2 to 9 mm. The gravel must be fine enough to hold sufficient moisture for the requirement of the plants, but at the same time, it should be coarse enough to allow free drainage. One of the merits of the gravel sub-irrigation system is 'old' air is forced out during pumping and 'fresh' air replaces it during drainage. Another advantage is that the solution can be reused for as long a period as it can adequately supply the plant's nutrient needs.

The tanks or troughs of a sub irrigation system are filled to near the rim with a nutrient solution between one and four times a day, depending on temperatures, day length and crop size. As soon as the trough is filled it is allowed to drain. The filling should be done by gravity as a pressurised water solution may disturb the medium and damage the roots.

Although gravel culture is the most efficient and practical type for commercial conditions, it also involves a major capital outlay. However, there is less wastage of water and nutrients, a greater control of the nutrient solution balance, and labour requirements are reduced. (HARRIS, 1987).

2.7.2.2 Non-recirculating systems (open systems)

These systems are generally used in conjunction with organic based media and the nutrient solution is run to waste after having wetted the medium. The nutrient solution used is generally the same as in the recirculating system. (A'BEAR, 1995, RESH, 1998).

2.7.2.2.1 Open bag system

This is the most popular system for the growing of long term crops such as cucumber, tomato, peppers and melons (HARRIS, 1987). The bags are usually 10 L black plastic type with drainage holes cut into the bottom of each container. The most commonly used media include composted bark, chopped oasis, perlite, palm peat, vermiculite, power station ash, gravel or coarse sand, coconut coir and rockwool .

The most important criterion in managing a bag system is that the nutrient solution at each watering must replace the remains of the nutrients from the previous watering. This means that up to 10% of the in-going solution must leach out from the bottom of the bag. It is not advisable to maintain too large a reservoir at the base of the bag as a high concentration of salt build-up will result. Judicious use of EC and pH meters are vital to ensure that the correct balances are maintained (HARRIS, 1987, A'BEAR, 1995, RESH, 1998).

Frequent watering of well draining medium is essential. Generally, pine shavings in 10 L bags need to be watered hourly. The amount of solution used depends upon such factors as crop season, daily temperature, bag size and stage of plant development. In summer, 2.5 to 3.0 L/plant/day could be required whilst in winter, 1.5 to 2.5 L/plant/day for a 10 L pot may suffice (A'BEAR,1995).

To allow for good drainage and effective hygiene the bags should be placed above a drainage channel. This reduces the risk of spreading diseases and limits the amount of free standing water. Ideally the floor should be plastic covered. Alternatively the channel can be filled with crushed stone. A gradient of 1:100 will allow for sufficient free drainage (A'BEAR, 1995).

2.7.2.2.2 Closed bag system

This system is widely used overseas in the form of rockwool slabs enclosed in plastic or 'tubes' of coir or palm peat. The cost of coir and rockwool can be prohibitive, though they can be used for up to five years. Overseas growers have tended to use rockwool slabs, but due to the problem of disposal (rockwool is not biodegradable) there is a strong move towards coir/coco peat. These bags are generally 1 m long, 10 to 15 cm thick and 20 cm wide. Holes are cut into the top of the bags at the required spacing and the plants are placed into the medium. Each plant is watered by its own watering tube. Drainage holes are cut into the bottom of the bags and bags are laid end to end next to a drainage channel (A'BEAR, 1995, RESH, 1998).

2.8 Cropping in inert media

2.8.1 Introduction

There are numerous types of media used in aggregate hydroponic systems. Inert products that have been used, singly or combined, include rockwool, perlite and vermiculite. Two of the most popular media for the growing of row crops such as tomato, cucumber and pepper are perlite and rockwool. When perlite and rockwool are used in closed systems, great care must be taken to avoid the build-up of toxic salts and to keep the system free of nematodes and soil borne diseases (JENSEN, 1999).

All these sterile inert materials are manufactured in a similar manner and share similar physical and chemical characteristics. They offer low cation exchange and large water-holding properties. They permit adequate root aeration and a high degree of control over watering and feeding. Furthermore, they allow energy savings for two reasons: 1). they eliminate soil steaming; 2). their use makes root heating practical. The latter allows for more precise control of air temperature on the basis of minimum temperature requirements of the shoots rather than the roots (JENSEN, 1999).

2.8.2 Vermiculite

Vermiculite, a hydrated aluminium-iron-magnesium silicate, appears in nature as plate-like crystals. Large deposits of this mineral occur in the United States and South Africa. When heated to approximately 1000 °C, the water trapped in it vaporises and causes the mineral to exfoliate. The plates or layers expand to 15 to 20 times their original volume, giving a lattice-like structure. The exfoliated vermiculite is a lightweight material of alkaline reaction, with high cation exchange and water-holding capacities (BUNT, 1988).

The material is available in a number of grades, ranging from a fine particle grade for seed germination, up to a grade with particles 6 mm in diameter. The average density is 80 kg/m^3. Vermiculite has a cation exchange capacity of 100 to 150 meq/100 g and compares favourably with peat (BUNT, 1988). Most samples contain 5 to 8% available potassium and 9 to 12% magnesium; mixes containing vermiculite therefore require less of these minerals in the base fertiliser.

A comparison of vermiculites from the USA and South Africa showed that the African vermiculite was more alkaline than the American material, the pH being 9.3 to 9.7 and 6.3 to 7.8 respectively. The African vermiculite had much higher magnesium levels: the calcium : magnesium ratio as measured by a Morgan's extract was 1:2 compared with a 6:1 ratio for the

American product. It was concluded that the African vermiculite was not detrimental when mixed with peat, but if lime was required a calcitic rather than dolomitic limestone should be used. If the vermiculite is not to be mixed with a low pH peat, a phosphoric acid drench at 0.68 ml of 85% phosphoric acid/100 g of dry Palaborwa vermiculite (grade three) is recommended (BUNT, 1988).

Vermiculite does not adsorb the anions Cl^-, NO_3^- and SO_4^{2-} but it does adsorb some PO_4^{3-}. When vermiculite was treated with a solution of potassium dihydrogen phosphate, 63 to 77% of the phosphorus was adsorbed. Only 25% of the adsorbed phosphorus was retained in an available form and 75% in a fixed form. The phosphorus formed insoluble compounds with sesquioxides and magnesium. Vermiculite is able to 'fix' large quantities of ammonium in an unavailable form. Most of the 'fixed' ammonium is, however, available to bacteria and is converted to nitrate within a few weeks. It then becomes available to plants. When vermiculite is used by itself as a growing medium for long term cropping, there is a tendency for the lattice or honeycomb structure to collapse, resulting in reduced aeration and drainage. For this reason it is advisable to mix either perlite or peat with the vermiculite (BUNT, 1988).

2.8.3 Perlite

According to LEE *et al.* (1999), the utilisation of perlite as a substrate for horticultural crop production under protected facilities was originally developed by Hall, Wilson and McGregor in 1984, for the production of tomatoes. Perlite is a grey-white mineral of volcanic origin mined from lava flows. It is a sterile, chemically inert, porous substrate having a neutral pH, low cation exchange capacity, negligible nutrient ion content, and a low content of heavy metals and other toxic materials. It has no buffering capacity and contains no mineral nutrients. Irrigation water is retained rather superficially and released at relatively low moisture tensions. It has a good capillary function which is a good physical characteristic especially in relation to air and water supply.

BUNT (1988) describes perlite as an alumino-silicate of volcanic origin. The USA and New Zealand have large natural deposits. When crushed and heated rapidly to 1000 °C, it expands to form white, lightweight aggregates with a closed cellular structure. These aggregates are stable and do not break down in a potting mixture. The average density of perlite is 128 kg/m^3 and it is available in a range of graded particle sizes. Because of its closed cellular structure, water is retained only on the surface of the aggregates or in pore spaces between them. This results in excellent drainage and low hydration requirements. Tests have shown that the volume of water present between container capacity and the wilting point is 75% for perlite and

42% for vermiculite. Plants grown in perlite prefer a capillary watering system to frequent hand watering. It is general experience that mixtures containing perlite are well aerated and have a lower available water content. Perlite is often mixed with peat and used as a rooting medium for cuttings, because its open structure prevents the occurrence of water-logging from the frequent mist applications in a greenhouse. The low bulk density of the mixture also means that the delicate roots are not so easily broken off during handling.

Perlite has virtually no cation exchange capacity having a value of only 1.5 meq/100 g. It is composed mostly of silicon-dioxide (73%) and aluminium-oxide (13%) and for practical purposes is devoid of plant nutrients. Plants grown in pure perlite are very dependent upon liquid feeding. Carnations grown in perlite suffer from aluminium toxicity when the pH of the nutrient solution is below 5.0, above this value no toxicity has been observed. Perlite contains small amounts of fluoride (17 mg/kg), and certain plants are susceptible to this element (BUNT, 1988).

PAPADOPOULOS (2000) states that perlite is widely used in the bag- and gulley-reservoir methods pioneered in Scotland. The bag-reservoir method is based on 30 L polyethylene growing bags (bolsters) filled with perlite and supporting three plants each. Its key features are the horizontal slits cut about 3 to 4 cm up from the base of the bag. In this open system, any excess nutrient solution applied collects to a depth of 3 to 4 cm in the bottom of the perlite in each bag. Aided by the high capillary action of perlite, this reservoir ensures an uninterrupted water supply to the plants with just a few irrigations per day and without placing a high demand on the irrigation system for uniform water delivery. The gulley-reservoir system is a closed system better suited to nutrient recirculation. The distinguishing feature of this system is the use of an extra sheet of plastic wrapped around the growing bags. This plastic maintains an even larger reservoir of nutrient solution or even allows for sub-irrigation to be practised when the greenhouse floor is reasonably level.

2.8.4 Sand and gravel

Sand is strictly defined as a particle in the size range 0.02 to 2 mm (Table 2.26). From numerous tests, it has been shown that sands with a high silt and clay content cause a decrease in porous spaces which results in poor gaseous exchange in the sand layer and insufficient oxygen supply to the root zone (SUZUKI, 1984). Natural sands generally contain various elements and if a root zone is growing soundly, it can draw essential micro-nutrients from the sand particle surface.

Table 2.26 Classification of soil by particle size

Class	Particle size
Gravel	> 2 mm
Coarse sand	2~0.2 mm
Fine sand	0.2 ~ 0.02 mm
Silt	0.02 ~ 0.002 mm
Clay	< 0.002 mm

(SUZUKI, 1984)

Sand and gravel are seldom used by themselves as the basis of a mixture and are usually in an admixture for the purpose of changing the general physical properties, e.g. the bulk density and water retention. Sand weighs approximately 1600 kg/m^3 and this can be an advantage in the case of tall plants, which have a tendency to topple over. Provided they are free from clay and calcium carbonate, sand and gravel have no effect upon the chemical characteristics of the medium. The difference between sand and gravel is purely one of particle size. Sand and gravel have an equally high yield potential when managed properly and can prove to be the best choice under certain conditions (BUNT, 1988).

An important point to observe when selecting sand is its freedom from carbonates. These will cause a large rise in the pH of the medium and create nutritional disorders, primarily affecting the availability of micro-elements, especially boron and iron.

WRIGHT (1992) describes sand culture as one of the most efficient and cost-effective methods of soilless culture. Its simplicity and low capital cost make it an attractive alternative to existing growing methods. Sand culture re-emerged during the 1960s, in Puerto Penasco, Mexico, where successful trials led to commercial operations in Mexico, Southwest USA. and in the Middle East. Using coarse beach sand, leached free of excess salts, vegetables were either sown directly in the sand or planted as seedlings.

An advantage of sand is its excellent capillary action, which results in lateral movement of nutrients and an even distribution throughout the root zone. Water retention is high owing to the smallness of the sand particles, allowing fewer irrigation cycles during the course of the day. Unlike other systems, particularly NFT, in the event of a fractured pipe, power or mechanical failure, there is more time available to repair the system before plants consume existing water in the medium and begin to experience stress due to dehydration. Practical advantages of sand culture include lower construction costs, simplicity of operation and ease of maintenance. Disadvantages of sand culture include the need to use chemical or steam

sterilisation between crops in order to destroy media-borne pathogens. Salt build-up is another common problem, but this can be corrected by flushing the medium periodically with fresh water (WRIGHT, 1992).

JENSEN (1999) curiously states that gravel is not recommended for use as an aggregate in either a closed or open system. This is in conflict with A'BEAR (1995) and RESH (1998) who regard gravel culture as the most widely used commercial method of hydroponics.

2.9 Recirculating hydroponic systems and disease susceptibility

2.9.1 Introduction

Disinfection of water to kill potential pathogens is necessary where it has originated in water courses, dams or has been recycled within farms or nurseries. Of greatest concern is the presence of *Phytophthora* species as they attack a wide range of plants and can affect both young and old plants. Many other pathogens attack a narrower range of plants or may attack only young seedlings or cuttings (MEBALDS *et al.*, 1997).

2.9.2 Sterilising recirculated water and nutrient solutions

There are a number of different types of sterilising techniques. They include slow sand filtration, heat treatment, ozonisation, UV-treatment, ultra-filtration, chlorine dioxide and chloro-bromination treatments (MEBALDS *et al.*, 1997). The quantity of solution recirculating through a NFT system for one hectare of tomatoes is about two million litres per day. The run off from one hectare of rockwool of tomatoes is about 20 000 litres per day (1% of NFT). Media-based systems like rockwool are being modified to collect the run off and return it for sterilising and reuse (WOHANKA, 1992, MEBALDS *et al.*, 1997).

2.9.3 Types of sterilising systems

2.9.3.1 Chemical sterilisation

Chlorine dioxide (ClO_2) is known to eliminate *Phytophthora cinnamomi* zoospores, spores of *Colletotrichum capsici* and *Fusarium oxysporum*. A concentration of 8.9 mg/kg chlorine dioxide with a four minute contact time is required to control chlamydospores of *P. cinnamomi*, compared with 2.6 mg/kg for two minutes needed to control the zoospores. *Pythium ultimum* can be eliminated at 1.2 mg/kg chlorine dioxide for two minutes in tap water and 2.4 mg/kg chlorine dioxide for four minutes in dam water. *Alternaria zinnae* is controlled in tap water with 3.1 mg/kg chlorine dioxide after eight minutes contact time (MEBALDS *et al.*, 1997).

MEBALDS *et al.* (1997) showed that applications of chlorine-bromine (Cl/Br) solutions to both dam and tap water resulted in an immediate draw-down of approximately 1 to 2 mg/kg available chlorine-bromine. The chlorine and bromine components were measured after addition to water to allow draw-down to occur and residual to be measured. The results showed that *P. cinnamomi* chlamydospores were not controlled in neutral tap water at 2.2 mg/kg total available chlorine-bromine (0.4 mg/kg Cl &1.8 mg/kg Br) or in alkaline dam water (pH 8.5 to 9.0) with 2.8 mg/kg total available chlorine-bromine (0.5 mg/kg Cl & 2.3 mg/kg Br),

but were controlled in neutral dam water (pH 6.5 to 7.0) after four minutes contact with 2.9 mg/kg available chlorine-bromine (1.0 mg/kg Cl & 1.9 mg/kg Br) and alkaline dam water (pH 9.4) with eight minutes contact at 8.0 mg/kg chlorine-bromine.

Chloro-bromination controlled *Pythium ultimum* with 2.2 mg/kg total available chlorine-bromine in tap water and eight minutes contact time or at 1.5 mg/kg total available chlorine-bromine in dam water with eight minutes contact time. Control of *Fusarium oxysporum* required similar doses in dam water but only 1.2 mg/kg total chlorine-bromine for four minutes in tap water. *Alternaria alternata* conidia were the most resistant spores; only 90% of spores were killed at 3.7 mg/kg total chlorine-bromine in tap water and 3.4 mg/kg total chlorine-bromine in dam water (MEBALDS *et al.*, 1997).

2.9.3.2 Ozone

RUNIA and AMSING (1996) describe ozone as a most powerful oxidising agent. As a donor of electrons (oxidation) to other substances, ozone itself is reduced to oxygen. The possibility of the interaction between reduction and oxidation is determined by the concept of redox potential, which is expressed in volts. The maximum ozone concentration in water is achieved at a redox value of 754 mV. Studies with ozone showed a rapid, natural decrease of dissolved ozone in water which varied between treatments when generation rate, gas and water flow rates were the same. The temperature and pH of water were found to influence the amount of dissolved ozone. The differences in residual ozone, ozone decay and ozone dose indicated that even when physical settings for ozone generation and dissolution are kept constant, actual doses vary. Furthermore, the rapid decay of ozone in water is non-linear and therefore average dose values are difficult to calculate in an 'on-site' situation. Analysis of water for ozone concentration can be easily performed using field spectrophotometers and pre-packaged reagents.

Ozone was able to control the chlamydospores of *P. cinnamomi* at an average dose of 0.6 mg/kg over 16 minutes. As the decay of ozone is logarithmic, a dose of 0.6 mg/kg for 16 minutes indicates an initial dose of around 1.2 to 2.4 mg/kg. Similarly, oospores of *Pythium ultimum* in tap water were controlled with an average dose of 1.2 to 1.5 mg/kg ozone and four minutes contact time. For dam water, an average dose of 0.4 to 0.7 mg/kg for eight minutes was required. Control of *Fusarium oxysporum* conidia in dam water was achieved after four minutes contact time with an average of 1.75 mg/kg residual ozone. The thicker spores of

Alternaria zinniae were more resistant to the ozonation process with only limited control with 1.3 mg/kg residual ozone and a contact time of 16 minutes (MEBALDS *et al.*, 1997).

2.9.3.3 Ultra Violet light

CARRUTHERS (1998) reported that ultraviolet (UV) light was widely used as a water disinfectant around the turn of the century, but its use declined with the introduction of cheaper alternatives such as chlorination. Over recent years there has been a renewed interest in UV-radiation as an alternative to chlorination, largely as a result of concern over toxic chemical by-products. UV-radiation treatment is unique in its mode of action in that it does not necessarily kill the target micro-organism. UV-radiation alters the DNA strands so that the micro-organism is sterilised, thus inactivating the pathogen so that it cannot proliferate and cause disease.

Germicidal UV-radiation is considered lethal for most micro-organisms, including bacteria, fungal spores, viruses, protozoa, nematode eggs and algae. The UV-light spectrum known to kill or deactivate most pathogens is between 100 to 280 nanometers. While pathogenic bacteria are the easiest group to treat, and differ the least in the amount of UV-radiation required, viruses are more resistant and variable in the amount of UV-radiation needed to 'neutralise' them. Some pathogens deactivated by UV-light may be reactivated when exposed to sunlight (CARRUTHERS, 1998).

Muddy water is known to reduce the effectiveness of UV-sterilisation, since the UV-light is easily absorbed by fine, solid particles and organic matter suspended in the water. UV-light can also be absorbed by deposits on the lamp sleeve. For hydroponic applications, especially where dam and borehole water is used, UV-units are often combined with carbon filters, membrane filtration and reverse osmosis systems, to achieve adequate water clarity (CARRUTHERS, 1998).

Recent research from The Netherlands recommends that the UV-radiation dose to control 99.9% of pathogenic bacteria and fungi is 100 mJ/cm^2, and the dose to control viruses is 250 mJ/cm^2 (CARRUTHERS, 1998). It has also been established that a minimum transparency level of 20% is needed for UV-treatment to be successful. The transmission value of water was calculated as the percentage of spore-killing UV-radiation after being passed through a 10 mm layer of water. The lower the value, the more energy required to achieve the correct UV-radiation dosage.

The transparency of drainage water from crops grown in rockwool is usually between 20% and 40% (CARRUTHERS, 1998). Where organic substrates are used, humic acid is released which lowers the transmission value of the water. The acids absorb a proportion of the UV-radiation and leave less available for sterilisation. This is also the case with iron chelates. In recirculating systems used for growing vegetables, the recommended level for iron in drainage water is 25 μmol/L.

The Research Station for Flowers and Glasshouse Vegetables at Naaldwijk, The Netherlands record that a dose of 100 mJ/cm^2 controlled *Fusarium* at 14% transparency. At 8% transparency, 110 mJ/cm^2 was needed and at 4% transparency, more than 174 mJ/cm^2 was required (CARRUTHERS, 1998).

2.9.3.4 Slow sand filtration

WOHANKA (1992) and RUNIA *et al.* (1996) indicate that recirculating systems generally include a considerable risk of spreading root pathogens. The nutrient solution, passing the root systems of diseased plants, might be contaminated with pathogens. In The Netherlands, some highly efficient methods of drain water disinfection, such as heating or ozonation, have been developed. These systems require a major capital injection and energy consumption costs are high. Viable, cheaper alternatives have been sought in Germany for the smaller scale operation.

Slow sand filtration is one of the oldest water treatment processes and was developed more than 100 years ago (WOHANKA, 1992, RUNIA *et al.*, 1996). It greatly reduced the danger to human health associated with the use of polluted surface water sources. Solution passes very slowly through a bed of fine sand with flow rates in the order of 100 to 300 litres/hour/m^2 surface area. Soon after the filter process begins, a filter skin forms on the surface of the filter bed. It consists of organic and inorganic material and a wide variety of biologically active micro-organisms which break down the organic matter. This biological activity and other mechanisms extend through the upper layer of the sand bed to a depth of 40 cm. The minimum thickness of the filter bed should be 50 to 60 cm. Depending on the raw water quality, cleaning the filter bed will be necessary after a few weeks or months. This is done by scraping off only the top few centimetres. Therefore, the initial thickness should be 80 to 120 cm (WOHANKA, 1992, RUNIA *et al.*, 1996).

The purification efficiency of a slow sand filter depends to a great extent on the grain size and its distribution. The effective grain size or effective diameter (d_{10}) is the sieve opening through which 10% (by mass) of grains will pass. The uniformity coefficient (UC) is the ratio between the effective diameter and the sieve opening through which 60% (by mass) of the grains will pass (d_{60}). The effective grain size should be in the range of 0.15 to 0.30 mm and the uniformity coefficient (UC = d_{10}/d_{60}) lower than three. The silt content should also be smaller than 1% otherwise the sand has to be washed before being placed into the filter. The acid solubility should not exceed 5% after 30 minutes (WOHANKA, 1992, RUNIA *et al.*, 1996).

The reliability and simplicity of slow sand filtration, and its efficiency in eliminating *Phytophthora* and *Pythium* from recirculating nutrient solutions or drainage water make it a viable system. High efficiency has been observed against *Cylindrocladium, Verticllium dahliae*, *Thielaviopsis* and *Xanthamonas* bacteria. Pathology work with *Fusarium* sp. has demonstrated a 99.9% reduction rate of mitochondria (small resting spores) which were poorly filtered by early designs of sand filters. It is assumed by researchers that this level of efficiency is sufficient to prevent serious problems with the dispersion of *Fusarium* through recirculated filtered water. However, *Fusarium* mitochondria are more resistant to heat and UV-treatment than other pathogens and are most likely to be most poorly controlled by any disinfestation method (WOHANKA, 1992, RUNIA *et al.*, 1996).

VAN OS *et al.* (1996) tested numerous sand fraction sizes and flow rates, and found that neither the flow rate nor the grain size seemed to influence the physical (EC, turbidity and temperature) and chemical (chemical oxygen demand - COD, biological oxygen demand - BOD_5, NH_4^+, NO_3^-, pH and oxygen concentration) content of the nutrient solution dramatically.

BLISS (1996) and MEBALDS *et al.* (1997) give a variety of treatments for the control of water-borne pathogens (Table 2.27).

Table 2.27 Treatments for the sterilisation of hydroponic solutions

Treatment	Advantages	Disadvantages
Steaming to 70 °C	Wide spectrum, non-toxic, no residual action.	Costly, unless cheap heat available.
Micro-filtration < 2 μm	Wide spectrum, non-toxic, no residual action. No chemicals.	Low flow/KW or m^2 unsuited for plants. Maintenance difficulties. High costs.
Slow sand	No chemicals, cheap to install.	May not control *Fusarium sp.*
Ozone (O_3)	Environmentally acceptable.	Costly to set up. O_3 can be toxic.
Hydrogen peroxide (H_2O_2)	Environmentally friendly, limited residual.	Not effective on Legionella.
Ultra Violet radiation	No chemicals, environmentally friendly. Relatively cheap.	Suitable for control of *Pythium* and *Phytophthora*. Works better in quality water.
Chlorine dioxide (ClO_2) (from chlorite + HCl does not release HOCl).	Environmentally acceptable, broad spectrum, effective residual, low phytotoxicity. Works in poor quality water at high pH.	Costly to set up. Chlorate/chlorite toxicity required careful mixing.
Chlorine hypochlorus acid (HOCl) from CaOCl, NaOCl	Broad spectrum, effective residual, very cheap.	Phytotoxic, less effective >33 °C, pH > 7.5 effect NaOCl, forms haloforms and inactive chloramines.
Bromine (HOBr) hyobromous acid (from bromine water)	Broad spectrum, effective residual, active bromamines.	Forms haloforms, availability limited.
Nylate (BCDMH), bromo-chloro-dimethyl-hydantoin. Gives HOCl + HOBr = 560 g/kg available Cl	Active bromamines, broad spectrum, effective residual, non-hytotoxic, effective at pH > 8.	Very low solubility, requires 'erosion feeder', forms haloforms, costs 3 x chlorine. Cheap capital cost, expensive running cost.

(BLISS, 1996, MEBALDS *et al.*, 1997)

2.9.4 The importance of flow rate

BUGBEE (1996) states that most hydroponic systems have inadequate flow rates, which result in reduced oxygen levels at root surfaces. This stresses roots and can increase the incidence of disease. Oxygen is soluble only as a micro-nutrient, yet its uptake rate is much faster than any other nutrient element.

NFT was designed to improve aeration of the nutrient solution, but the slow flow rates in NFT can cause channelling of the solution and reduced flow to areas with dense roots. The root surfaces in these areas may become anaerobic, which will diminish root respiration, reduce nutrient uptake, increase nitrogen losses via denitrification, and make roots more susceptible to infection (BUGBEE, 1996).

2.9.5 Disease organisms and their effect on recirculating systems

COOPER (1996) reported that when the prototype of NFT was first being tried out, the majority of scientists believed that a disease organism would enter one channel and spread throughout the system via the recirculating solution resulting in the death of the whole crop. NFT cropping has been done either on a trial basis or on a commercial scale in at least 80 countries. There have been cases where dead and dying root systems have been reported and disease organisms identified, but these have nearly always been found to be secondary pathogens. Disease organisms have not established themselves until the root system has been adversely affected by factors such as phytotoxic components in the hardware, or by poor root growth resulting form a lack of aeration, due to the 'drowning' of the root system. The normal spraying methods of conventional disease control can be applied to an NFT crop (COOPER, 1996).

PRICE and NOLAN (1984) record that the NFT for growing plants has been adopted as a method of avoiding or overcoming losses due to soil-borne plant pathogens such as *Pythium* and *Fusarium* sp. Plants in NFT systems are fed constantly by nutrients and the addition of systemic pesticides to the nutrient supply has the potential of affording protection as well as providing substantial savings on labour and application costs.

COOPER (1996) offers one hypothesis as to why diseases have not rapidly spread as a result of recirculating a nutrient solution. Some soil-borne disease organisms require mechanical damage of the roots as an entry point. In soil, this mechanical damage is provided by damage to the root hairs as the roots move through the soil particles in the soil. In NFT channels there are very few root hairs and no solid particles to provide the mechanical damage.

RAHMAN (1984) reported that during the early 1970s NFT was tested on a small scale, particularly with tomatoes, by research and experimental stations, and a few commercial growers. A common factor arising out of reported trials was that severe wilting often occurred when plants were carrying a heavy crop of fruit. Wilting was associated with death of roots, the plants recovering at the onset of picking.

RAHMAN (1984) experimented with tomato plants in an NFT system. Some of the plants were grown with fruit and others without fruit, in order to assess the effect of fruiting on the presence of growth inhibiting substances in the nutrient solution. Fungi were also isolated from the nutrient solutions and the efficiency of activated charcoal as a filter to purify nutrients was also tested. Bioassay determinations using barley coleoptiles and lettuce hypocotyls were made weekly over 52 to 164 days from sowing. Nutrient solutions were adjusted to a conductivity of

3.0 mS/cm by addition of diluted stock solution. Assessment of fungi was carried out using P_{10} VP medium.

Pythium sp. (*Pythium ultimum* and *Pythium irregulare*) were found in all the solutions at least once, but with no clear pattern of occurrence. The data indicated that when the root system was poor, there was a lower amount of nitrogen in both shoots and roots. The smaller roots were also associated with low amounts of copper in the roots and shoots. Low uptake of nitrogen and copper could also be measured during the root death period. It was not clear whether changes in rate of uptake of copper and other elements by tomato roots grown in NFT, were associated with naturally occurring or artificially added populations of fungi (RAHMAN, 1984).

BUGBEE (1996) states that the *Pythium* fungus has been the only serious pathogen encountered in his experiments with NFT systems, particularly when all parts of the system were covered in order to keep dust and dirt particles away from the solution. A manganese deficiency predisposed the plants to *Pythium* infection. Copper is well known to suppress microbial growth, but increased copper levels are toxic to plants. Manganese and zinc (divalent cations) may have a similar disease suppressive potential, but are less toxic to plants.

Results from trials carried out on carnations by PRICE and NOLAN (1984), showed that *Fusarium* propagules can be dispersed through a NFT system through the use of chlorine. The rapid decline in the number of propagules recovered could not be accounted for either by adsorption onto the walls of the channels or onto the root surfaces. It is likely that the *Fusarium* propagules sediment with time in the reservoir.

Concentrations of 2.5 to 5.0 mg/l chlorine were sufficient to cause total mortality of *Fusarium conidia* within five minutes. The decline in chlorine concentration resulted in a reduction of fungicidal activity with time and indicated that daily chlorination would be necessary to kill any fungal propagules that may enter the system. Unfortunately, daily additions can also result in phytotoxicity often expressed as a potassium deficiency. An increase in sodium ions in the nutrients solution caused an imbalance in the ratio of sodium to potassium in plants (PRICE and NOLAN, 1984).

Additions of benomyl fungicide (50 mg/L) to a 40 L reservoir containing 2000 *Fusarium conidia* /ml inhibited the fungus. The fungicide was distributed throughout the circulating system and

remained there for at least 21 days. The benomyl was not toxic to carnations at this dosage nor did it affect the pH or levels of magnesium, iron, calcium, manganese, sodium or potassium in the solution (PRICE and NOLAN, 1984).

2.9.6 Effects of nutrients on eucalypt seedling diseases

In a glasshouse trial, seedlings of *E. maculata* and *E. pilularis* grown in sand and supplied with varied levels of nitrogen, phosphorus and calcium, were inoculated with *Phytophthora cinnamomi.* The response of the seedlings was evaluated nine weeks after inoculation (HALSALL *et al.*, 1983).

Seedling growth (height, shoot and root dry mass) increased with increasing levels of nitrogen and phosphorus. Increased calcium levels produced an increase in shoot mass only when phosphorus levels were high. High levels of both nitrogen and phosphorus produced a synergistic increase in growth although at the highest level of nitrogen (120 mg/l), there was a decrease in growth when phosphorus and calcium were also supplied at the highest levels. Phosphorus ceased to be limiting above 6 mg/l (HALSALL *et al.*, 1983).

Inoculation with *P. cinnamomi* caused a significant reduction in growth in both species of eucalypt. This reduction was highest when there was a nutrient imbalance between nitrogen and phosphorus and lowest when nitrogen and phosphorus levels were balanced and the plants were growing vigorously. The pathogen was recovered more frequently from seedlings growing on low levels of nitrogen and phosphorus indicating that *P. cinnamomi* is less successful in infection or survives in the root for a shorter period when nutritional conditions favour vigorous growth. The form of nitrogen supplied also affected seedling growth. Maximum growth was obtained when the nitrate to ammonium ratio was 7 : 3. The form of nitrogen supplied did not affect the response to inoculation with *P. cinnamomi* (HALSALL *et al.*, 1983).

2.10 The development of propagation technology in clonal forestry

2.10.1 Introduction

Mass vegetative propagation has become an important tool for increasing the competitiveness of the forestry based industry. Its greatest contribution can be achieved when it is utilised to establish clonal forests of hybrids endowed with superb fibre quality and higher volumetric growth. In the eucalypts, the popular method of rooting stem cuttings has limitations such as the rapid loss of rooting competence due to ontogenetic ageing, intra-clonal variation resulting from topophysis, and poor quality root systems that negatively affect the genetic expression of superior clones (DE ASSIS, 2001).

Two intensive propagation systems have developed for the cloning of eucalypts on a commercial scale:

- 'Micro-cutting' system - utilising the apices obtained from micro-propagated plantlets.
- 'Mini-cutting' system - utilising axillary bud sprouts from rooted stem cuttings.

In both systems the ramets are managed intensively to produce mini-cuttings. Conventional field hedges can thus be replaced by indoor hydroponic 'mini-hedges', which provide a higher level of juvenility. The success of the systems are strongly dependent upon optimal nutrient status of the 'mini-hedges' (DE ASSIS, 2001).

BONGA (1982) indicates that the capacity to vegetatively propagate trees is associated with juvenility. Generally, the more juvenile the specimen, the easier it is to propagate vegetatively. There is no clearly defined transition from the juvenile to the mature phase in most plants. Often, some parts of the tree may be mature, or senescent, while other portions still display juvenile characteristics. Furthermore, in some trees, major morphological characteristics may change abruptly (heteroblastic) when maturing, while in others, the changes are more gradual (homoblastic).

The development of these intensive cloning systems has set the stage for a new phase of mass vegetative propagation of eucalypts and other woody species. Compared to stem cuttings from existing hedge systems, the rooting of micro- or mini-cuttings improves rooting potential, rooting speed, root system quality and reduces production and maintenance costs.

2.10.2 The development of the mass production of vegetative propagules

CAMPINHOS and IKEMORI (1983) record that the precursor of this new clonal technique had its origins in the 1980s when cloning of *Eucalyptus* by rooting stem cuttings reached a commercial dimension. The rooting of stem cuttings had proven to be unsuccessful for a number of economically important eucalypt species, and a number of clones that could be clonally propagated had problems associated with accelerated maturation and a subsequent decrease in rooting ability. The phenomenon of topophysism was the main cause of intra-clonal differences in growth and reduction of rooting ability. HARTMAN *et al.* (1990) defines topophysis as the effect of the position on the plant of the propagule on the type of vegetative growth subsequently shown by the vegetative progeny.

The first vegetative cuttings for the planting of clonal forests were obtained from plantations. This management strategy required the annual reservation of large areas for the sole purpose of coppice collection. Although this method could produce the propagules, the high demand for timber made it unfeasible. The clonal hedge system concept of intensive management was introduced sometime later. Despite being a tremendous advance for the mass production of shoots, clonal hedges are complicated to manage and poorly controlled. To meet the demands of clonal forestry, the system still requires large areas, intensive labour management and large quantities of fertiliser and water. The propagators are inevitably reliant on the prevailing weather since the system is at the mercy of environmental conditions (CARVALHO *et al.*, 1991, HIGASHI *et al.*, 2000).

Another limitation of the stem cutting can be associated with alterations of the root architecture, leading to root deformation. In many clones such deformations prevent their full genetic expression, reducing the ratio between selected trees and the number of clones effectively deployed. Because of the limitations of stem cuttings, alternative methods have been sought and developed for the commercial cloning of eucalypts (CAMPINHOS and IKEMORI, 1983).

2.10.3 The micro-cutting system

Based on the work of DE ASSIS *et al.* (1992), the micro-cutting technique was developed in Brazil in the early 1990s. The idea came from observations that the rooting of stem cuttings decreases with ontogenetic ageing and that the decline in rooting may be more rapid than previously reported. PATON and WILLING (1974) reported that in *E. grandis*, the rooting competence decreased from the fourteenth node up. DE ASSIS *et al.* (1992) observed that clones of *E. saligna, E. grandis* and *E. urophylla* that had equally high proportions of stem

cutting rooting *in vitro,* showed differential levels of decline in rooting percentages when managed in clonal hedges. This indicated that some factor related to ramet growth, encompassing the period between planting and harvest of cuttings (six months), could be responsible for these differences.

Preliminary tests carried out at Klabin Riocell (unpublished) produced, irrespective of the species, almost 100% rooting of young mini-cuttings obtained from the cotyledonary leaf pair. This rooting ability was maintained in difficult to root species like *E. citridora, E. cloeziana, E. paniculata, E. dunnii* and *E. globulus.* However, with age, ranging from a few days to some months, the cuttings harvested showed a marked reduction in their rooting ability. DE ASSIS (2001) suggests that the rooting potential reaches a maximum value at the high juvenility stage (mini-cuttings from cotyledons) irrespective of species. However, the decrease in the rooting ability with seedling age differed among species, which was similar to that found in older material in the field. This suggests that, at some stage, part of the juvenility inherited through the rejuvenation process *(in vitro* or on basal sprouts of felled adult trees) is being eroded through the growth of ramets in clonal hedges.

WILSON (1998a) offers an interesting but conflicting opinion and suggests that much of the loss of 'rooting ability' ascribed to physiological ageing or maturation is in fact due to an increase in mortality of cuttings. Adult morphology of cuttings tend to have relatively low pre-severance growth rates, little or no post-severance growth potential (expansion of immature leaves, axillary shoots, callus development), high rates of leaf abscission and probably a low ability to take up water through the stiff and strongly lignified stem.

DE ASSIS (2001) notes that theoretically, the rooting ability of *Eucalyptus* clones *(ex vitro)* should increase if the 'physiological distance' between the maximum juvenility stage (obtained *in vitro*) and the propagule collecting stage is reduced. To test this hypothesis DE ASSIS *et al.* (1992) used the shoot apices (micro-cuttings) of very juvenile micro-propagated plants of an *E. saligna* clone as propagules. These micro-cuttings had a 30% higher rooting than the stem cuttings. These findings were re-tested on seven clones of *E. saligna* and five clones of *E. grandis.* On average, the rooting of micro-cuttings was 18% higher than stem cuttings.

One of the most significant findings of this new technology was the complete elimination of the use of rooting hormones usually required for the rooting of stem cuttings. These hormones proved not only to be extraneous, but in some cases actually reduced rooting, indicating that

the endogenous auxin concentration in the juvenile tissues was sufficient to promote rooting. (DE ASSIS *et al.*, 1992).

DE ASSIS (2001) describes the primary feature of the new technique as the utilisation of juvenile plants or plants rejuvenated *in vitro,* as a source of vegetative propagules. Shoot apices are used as micro-cuttings, which are rooted in a glasshouse having accurate temperature and humidity control. The micro-cuttings are 7 to 8 cm with two to three leaf pairs. The presence of a **shoot apex** is essential for the development of a quality root system, as its presence induces a taproot-like system. The micro-stumps sprout extremely rapidly producing new propagules, which can be harvested within a period of 15 days in summer and 30 days in winter.

2.10.4 The mini-cutting system

DE ASSIS (2001) reports that the first attempts to root mini-cuttings of *Eucalyptus* were in the early 1980s, by using shoots obtained from the thinning operation of rooted stem-cuttings. Early trials were discouraging due to the inconsistent results. Later studies showed that such inconsistencies were as a result of nutritional deficiencies of the mother plant at the time of cutting collection. In the 1990s, after the consolidation of the micro-cutting as a functional propagation system, the mini-cutting system became commercially viable for *Eucalyptus* cloning. HIGASHI *et al.* (2000) developed a functional and efficient mini-cutting system and several other researchers have contributed to its streamlining.

Mini- and micro-cutting techniques are very similar in concept and operational procedures, differing mainly in the origin of the initial propagules (DE ASSIS, 2001). Micro-cuttings are obtained from shoot apices originating from micro-propagated plants, whilst mini-cuttings originate from axillary sprouts of plants cloned from stem-cuttings (DE ASSIS, 2001). After the rooting of the first shoot, the two techniques become identical. In some clones using the mini-cutting technique, a number of propagation cycles (serial propagation) are required to reactivate the full rooting potential. In micro-cuttings, such propagation cycles come naturally by monthly *in vitro* sub-culturing of explants. Micro-propagation is unnecessary for easy-to-root species as high levels of juvenility can be obtained by inducing basal shoots. In such cases mini-cuttings are more economically feasible (DE ASSIS, 2001).

Although the success of these techniques has been attributed to the maintenance of a very juvenile stage, new findings indicate that their high rooting potential is also related to the better

nutritional status of the mini-cuttings (DE ASSIS *et al.*, 1992, XAVIER and COMÉRIO, 1996). In general, mini-cuttings or micro-cuttings root better than juvenile stem cuttings of the same clone harvested from field hedges, as a result of the hedge plant's improved nutritional status. This improved rooting may also be due to differential levels of lignification in the two groups of propagules. Compared to stem cuttings, micro- and mini-cuttings can be considered 'herbaceous', and by using these techniques many complications associated with lignin formation and its concentration increase in tissues can be avoided. DE ASSIS (2001) explains that the two techniques are very similar, and that the terms designated (mini-cutting and micro-cutting) are by convention only. Thus micro-cuttings originate from micro-propagated plants, whilst mini-cuttings are based on sprouts from rooted cuttings. There is a tendency to change this terminology to avoid confusion with the traditional term 'micro-cutting' reserved for rooting of shoots produced ***in vitro*** (Figure 2.13).

Figure 2.13 Micro-propagated *E. nitens x E. grandis* hedge plants in the ebb and flow system at Mountain Home Nursery. *In vitro* propagated E.GxN clones show more seedling-like qualities with a superior fibrous root system and dominant apical shoot

2.10.5 Advantages of the systems

In comparison to the traditional stem cutting, the mini-cutting has a number of advantages. Operationally, the labour demand and cost thereof is markedly reduced due to the elimination of labour intensive practices associated with conventional field hedges. Cultural practices such as soil preparation, fertilisation, irrigation, cultivation, weeding, pest and disease control, are replaced by intensive activities in a much smaller area at much lower costs, where the amount of chemicals used is drastically reduced (DE ASSIS, 2001).

The rooting ability of mini-cuttings is generally much higher than stem cuttings although results can vary by clone and species. The main reason for improved rooting is the higher levels of juvenility and optimal nutritional content of the tissues. The rooting speed of mini-cuttings has two other important consequences in a commercial cloning programme:

1. The period that the plants remain in the greenhouse is reduced by up to half as compared to a stem cutting, thus considerably improving the use of the structure; and
2. Reduction in the time that the basal tissue is exposed to pathogenic fungi.

At the initial and most susceptible stage, the faster reaction of the mini-cuttings to inducing rapid protector callus formation at the basal end, provides protection from pathogens resulting in reduced fungicide application (DE ASSIS, 2001).

Mini-cuttings produce a better quality root system with a tendency for a taproot-like system in contrast to the predominant lateral root growth habit of stem cuttings. This kind of root formation is more responsive to fertilisation, more resistant to adverse environmental factors and greatly reduces intra-clonal variation.

2.10.6 Mini-clonal hedges

At the beginning of the last decade, the development of micro-cutting technology for eucalypts accelerated the concept of a 'super-intensive' management system of producing vegetative propagules on a commercial scale. The system was initially based on mini-hedges established through mini-cuttings, grown in small tubes which provided a series of technical and economic benefits as well as good root quality (DE ASSIS *et al.*, 1992, XAVIER and COMÉRIO, 1996).

Despite being a great advance over field hedges, mini-hedges face a number of limitations. The outdoor mini-hedge is still at the mercy of fluctuating weather conditions, and the problems related to adequate nutritional status and leaf disease prevalence continue, especially during

winter. The main problems are reduced photsynthetic rates, reduced nutrient uptake and high levels of nutrient loss by leaching during periods of high rainfall. All these limiting factors have lead to the development of the indoor mini-hedge.

2.10.7 Indoor hydroponic clonal hedges

HIGASHI *et al.* (2000) record that one of the first hydroponic systems for the production of mini-cuttings consisted of sand beds and drip irrigation. The major contribution of the system was in terms of assuring adequate nutrient supply to the ramets, which is a key factor in obtaining high rooting percentages from juvenile vegetative material. Using the same concept, CAMPINHOS *et al.* (2000) developed a highly efficient method based on an intermittent flooding system, where the mini-stump containers were immersed in a solution. These two hydroponic methods, sand bed and intermittent flooding, are still the most widely used in Brazil (DE ASSIS, 2001).

2.10.8 Advantages of indoor hydroponic mini-hedging

According to DE ASSIS (2001), the development of the concept of hydroponic mini-hedging brought several benefits to the commercial cloning of *Eucalyptus*. Higher productivity of mini-cuttings, lower labour demand and low consumption of chemicals and water represent major savings (Figure 2.14). Furthermore, the system allows for CO_2 enrichment control, control of temperature, light intensity and photoperiod manipulation. These factors, along with optimum nutrition, are of fundamental importance in the enhancement of the rooting predisposition of clonal eucalypts. The system also allows for the foliar application of plant growth regulants to mother plants that may improve rooting potential.

Figure 2.14 The first indoor clonal mini-hedges in South Africa - Mondi Forests, Mountain Home Nursery

2.10.9 Propagule productivity

The first cloning of eucalypts on a large scale in Brazil used very extensive hedges with an output of 114 cuttings/m^2 (CAMPINHOS and IKEMORI, 1983). CARVALHO *et al.* (1991) note that with the introduction of mini-hedges, production increased to 121 cuttings/m^2 and HIGASHI *et al.* (2000) reported a further increase of up to 1752 cuttings/m^2. DE ASSIS (2001) states that the current production is 24000 cuttings/m^2. Table 2.28 is a summary of the increase in clonal cuttings productivity in Brazil.

Table 2.28 Propagule productivity in different clonal hedging systems in Brazil

System	Species and espacement (m)	Productivity (cuttings/m²/year)	References
Clonal bank	*E. grandis x E. urophylla* 3.0 x 3.0	114	CAMPINHOS and IKEMORI (1983)
Clonal hedging	*E. grandis x E. urophylla* 1.0 x 1.5	121	CARVALHO *et al.* (1991)
Clonal hedging	*E. grandis x E. urophylla* 0.5 x 0.5	1752	HIGASHI *et al.* (2000)
Mini hedging (outdoor)	*E. grandis x E. urophylla* 0.05 x 0.05	29200	XAVIER and COMÉRIO (1996)
Hydro mini-hedging (sand bed/ drip irrigation)	*E. grandis x E. urophylla* 0.1 x 0.1	41480 **	HIGASHI *et al* . (2000)
Hydro mini-hedging (intermittent flooding)	*E. grandis x E. urophylla* 0.05 x 0.05	25200	CAMPINHOS *et al.* (2000)
Hydro mini-hedging (intermittent flooding)	*E. grandis x E. urophylla* 0.05 x 0.05	24000	KLABIN RIOCELL (unpublished)
Hydro mini-hedging (intermittent flooding)	*E. saligna; E. grandis x E. urophylla* 0.05 x 0.05	14400	KLABIN RIOCELL (unpublished)
Virtual mini-hedges	*E. saligna; E. grandis x E. urophylla* 0.05 x 0.05		KLABIN RIOCELL (unpublished)

** Subdividing axillary sprouts into 3 mini-cuttings. (DE ASSIS, 2001)

Hydroponic based systems are much more efficient than conventional field hedges and have productivity outputs up to 350 times higher than the original systems first introduced in Brazil. Variations in the productivity of the mini-cutting hedges (Table 2.28) are as a result of different hedge systems, different genetic material and varying management practices. Clones of *E. grandis x E. urophylla* hybrids are generally more productive in mini-cutting hedges than any other eucalypt species. The majority of the pure *E. saligna* clones are much more difficult to root as mini-cuttings. However, the rooting ability and cutting productivity is much higher than in field hedges (DE ASSIS, 2001).

According to DE ASSIS (2001), certain variations of stump management are practised in sand beds that allow for the production of cuttings similar in size to the macro-cutting system. Each sprout or cutting is allowed to grow to a larger size and is then divided into three mini-cuttings (basal, middle and apical). This system produces higher numbers of propagules/m² but loses the best characteristics of the mini-cutting provided by the presence of a shoot apex.

In both hydroponic systems (drip fertigation and intermittent flooding), the loss of mother plants is common as a result of excessive harvesting, disease incidence and toxic salt build-up. The intermittent flooding system (ebb and flow) (Figure 2.14) has certain advantages over the sand beds such as the reduced time needed for replacement plants to become productive again.

Mother plants from the intermittent flooding system can be planted out in the field provided that the harvesting regime has not degraded its quality. Under successive and heavy harvesting regimes the quality of the mother plant frequently declines (DE ASSIS, 2001).

One concern that arises is the effect of close spacing of mini-hedges on the rooting of mini-cuttings and mini-stump production. Trials at Klabin Riocell showed that at an espacement of 5 x 5 cm there was a significant effect on rooting, but a wider spacing produced a higher survival rate of mini-stumps. The closer espacement allowed for a greater number of stock plants/m^2 which fully compensated for the lower survival rate (DE ASSIS, 2001).

CHAPTER 3

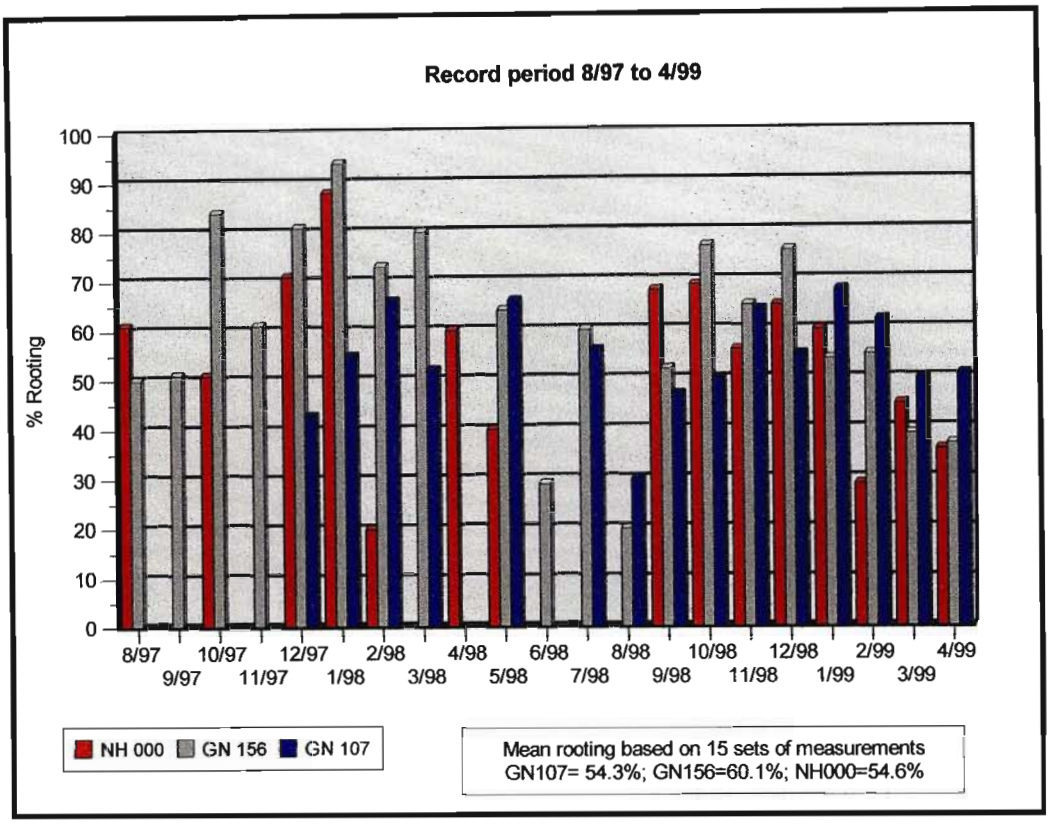
COMPARISON OF EXPERIMENTAL RECIRCULATING HYDROPONIC SYSTEMS

3.1 INTRODUCTION

It is generally advocated that the rooting potential of genetically improved eucalypt mother plants (stock plants) is strongly linked to their nutrient status. However, achieving and sustaining an optimum nutritional balance is very difficult in field hedges due to the complexities of the interacting edaphic factors. In order to accurately predetermine the optimum plant nutrition (required all year round, to ensure economic levels of rooting), a more controllable environment is essential. The cross, *E. grandis x E. nitens,* a prime example of a hybrid with outstanding volume yields and excellent fibre properties, eludes our abilities to propagate at economically sustainable levels. Hydroponics may facilitate the uniformity and control required, particularly in nutrition, aeration and CO_2 enrichment. At the same time it may be possible to manipulate the system to accurately determine what level and type of fertilisation will contribute most to improved rooting and more importantly, allow us to sustain that level.

Little has changed in the management of field clonal hedges since their first plantings some twenty years ago and protocols developed then are still generally applied. In order to produce eight to ten million plants annually, the Mondi clonal nursery at Kwambonambi (KwaZulu-Natal, South Africa) has to maintain 25-30 ha of clonal hedges. This scale of operation has massive logistical implications and is extremely labour intensive. Nurserymen have difficulty in accurately predicting expected cutting yield and root strike from clonal field hedges. Seasonal fluctuations are well recognised but little can be done to counteract their onset and effects on rooting performance (Figure 3.1).

Figure 3.1 Rooting percentages of test clones from field hedges at Mountain Home Nursery (rooting % measured on removal from greenhouse and not final survival figure)



The current system of extensive clonal hedges is difficult and costly to maintain. Root strike of field derived cuttings varies from month to month with a resultant inability to accurately predict plant availability. The success of a temperate clonal propagation programme hinges on removing these 'dips' and sustaining a consistently high level of rooting (75%).

Brazilian forest companies have proven that hydroponics and 'hydro-hedges' have a major role to play in the future clonal propagation of subtropical hybrid eucalypts such as *E. grandis x E. urophylla.* The challenge is to determine whether **all** of the important eucalypts currently planted in South African forests can be propagated from hydroponically sustained mother plants. This technology holds the potential to revolutionise clonal production capacities and improve the overall efficiencies of existing clonal nurseries.

This trial was designed to (a) aid in the development and comparison of two experimental, mobile, recirculating hydroponic systems suitable for sustaining *E. grandis x E. nitens* clonal stock plants and the possible effects on rooting performance and hedge plant sustainability; (b) to determine the most suitable substrate that could sustain optimum growth of hedge

plants and generate rooting responses in excess of 60%. A further objective was to assess the commercial application of the system(s) to clonal forest nurseries.

3.2 MATERIALS AND METHODS

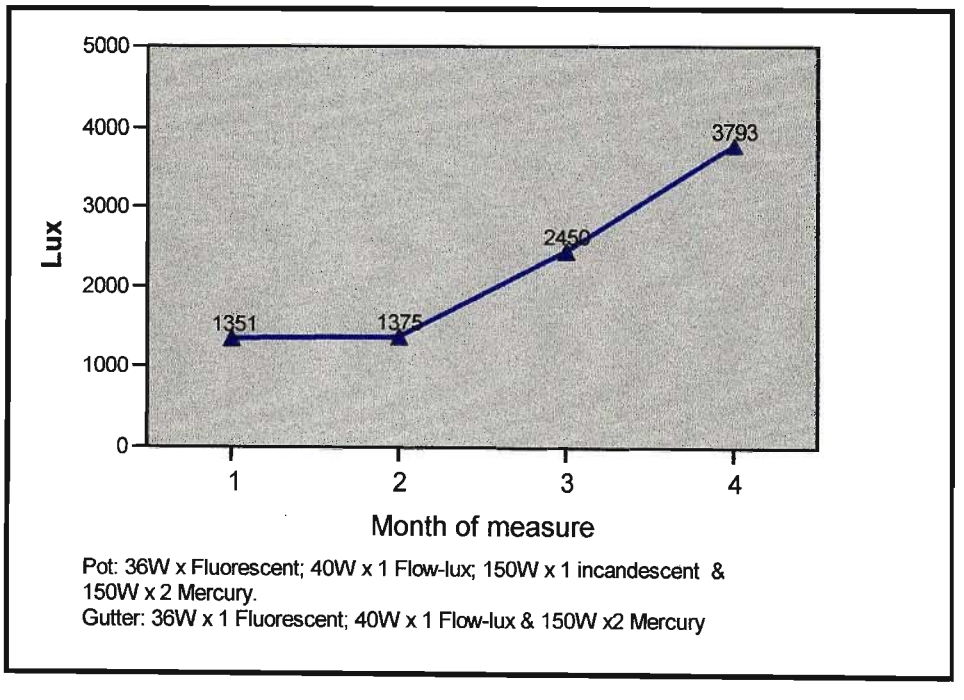
3.2.1 Systems design

The trial was established on 23/6/99 and all experimentation was carried out at the Mondi Forests, Mountain Home Nursery, KwaZulu-Natal. Two separate 'modified' NFT recirculating units were purposely designed and built. Both units were 3.0 m x 1.2 m in dimension and manufactured from 32 mm square mild steel tubing. The 'trough system' comprised 4 x 110 mm polypipe sections braced on to the frame whilst the 'pot system' consisted of 36 rigid plastic pots (volume = 1 L). The polypipe sections were lined with capillary matting to ensure the even distribution of liquid feed. Insufficient lighting required the installation of supplementary light fittings. The first three attempts at improving light intensity resulted in minor improvements from 1351 Lux to 2450 Lux (Figure 3.2). In the fourth month, each unit was reconfigured and the following globe combinations were installed:

- One 36 W new generation Gro-lux® fluorescent tube;
- One 40 W Flow-lux® tube;
- One 150 W incandescent bulb; and
- Two 150 W mercury globes.

This combination of lighting improved the intensity to 3793 Lux (Figure 3.2).

Figure 3.2 Enhancement of artificial lighting for experimental designs

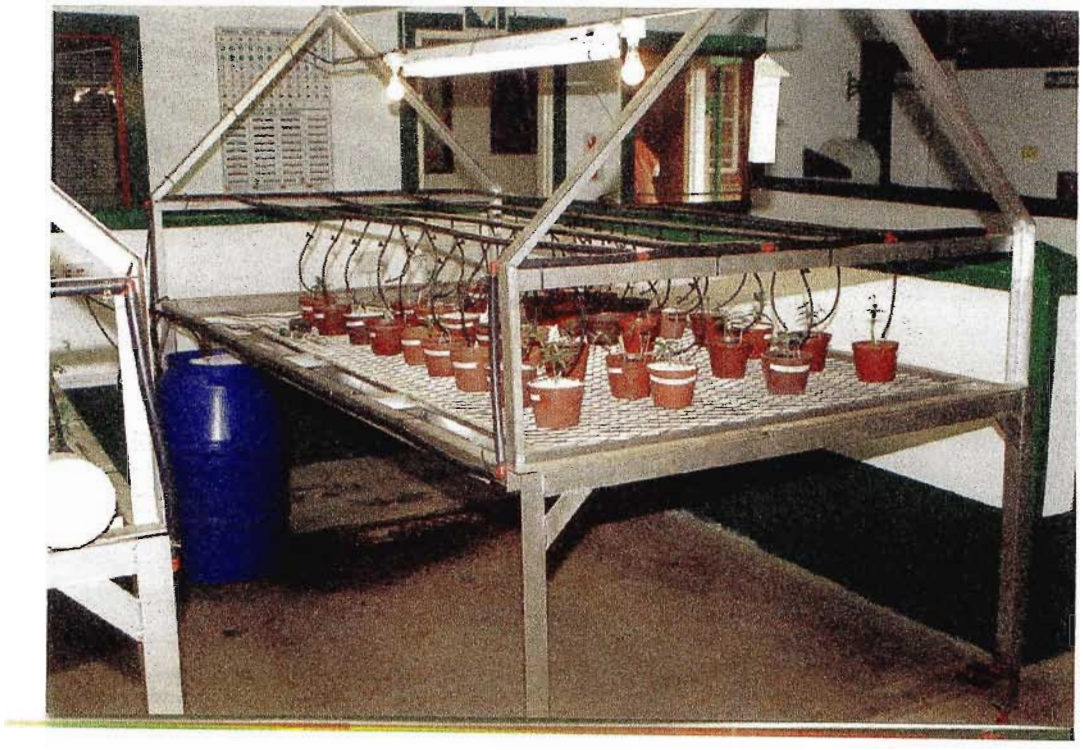


Each unit was irrigated via a submersible pump (maximum volume 1400 L/hr to a maximum head of 1.8 m) installed in a 180 L plastic tank. An additional pump unit was inserted into each tank to ensure proper aeration and dissolving of fertilisers (Figure 3.3 and Figure 3.4). Unequal cyclic timers were fitted to the individual units. Due to differing media volumes and the potential to over-saturate the pots, the unequal cyclic timer on the pot unit was set to run for six minutes with an off-time interval of thirty minutes. The timer for the trough unit was set to run for three minutes with an off-time interval of thirty minutes. Both timers were linked to an electronic clock that disrupted power supply at 18h00 and reconnected at 06h00.

WILSON (1998b) experimented with *E. globulus* potted mother plants and discovered that (a) supplementary lighting to prolong day-length in winter, and (b) periodic water stress resulted in an increase in the initial survival of cuttings harvested from them. Both treatments (supplementary lighting and water stress) reduced cuticular transpiration from cuttings since they visibly increased glaucousness. Stress may also have conditioned the stomata to close more readily prior to wilting. Glaucousness was a potentially adverse trait, reducing wettability under intermittent mist, but could largely be nullified if cuttings were briefly immersed in fungicide solution before setting. This treatment markedly increased survival ability and was accompanied by a conspicuous and virtually immediate colour change to a non-glaucous green.

Figure 3.3 Prototype of trough system using 110 mm polypipe (piping proved to be cumbersome and it was difficult to place plants. Note additional lighting)

Figure 3.4 Prototype of pot system using 1L plastic pots



3.2.2 Test clones

Three Mondi clones were selected for testing in the hydroponic units on the basis of their mean rooting performance (Table 3.1). These clones are no longer commercially planted and have been superseded by superior hybrid crosses. All ramets had well consolidated root plugs at planting. All ramet lengths were measured and every effort was made to select plants of similar length. The planting lengths are recorded (Appendix C1.1 and C1.2).

Table 3.1 Rooting statistics of selected clones from field hedges, 8/1997 to 4/1999

Summary Statistics	NH000	GN156	GN107
No. values	15.00	20.00	15.00
Mean rooting	54.6%	60.1%	54.3%
Median	60.00	60.50	55.00
Minimum	20.00	20.00	30.00
Maximum	88.00	94.00	68.00
Range	68.00	74.00	38.00
Variance	321.26	377.15	103.10
Standard deviation	17.92	19.42	10.15
Standard error of mean	4.63	4.34	2.62
Coefficient of variation	32.83	32.31	18.69
Skewness	-0.26	-0.28	-0.66

3.2.3 Hedge plants as controls

A field clonal control block of the three clones was planted on 30/6/99 and cut back in April 2000. Control blocks of 25 (five x five) plants were established and demarcated. The blocks were established at an espacement of 50 cm x 60 cm and fertigated with orange fertiliser at 500 µS/cm, twice a week (see Appendix C). A leaf analysis was completed when other material was submitted for analysis (Table 3.5). Soil samples were sent for analysis to the soil fertility and analytical services section at Cedara. The predominant soil was identified as an 'Inanda' type. Eight random samples were sent for analysis. Mean data for the soil type is presented in Table 3.2.

Table 3.2 Analysis of clonal hedge soil samples

pH (KCL)	P mg/L	Mg mg/L	K mg/L	Ca mg/L	Zn mg/L	Mn mg/L	Acid sat %	Total cations cmol/L	Sample density g/ml	Organic carbon %
4.22	5.6	19.88	31.5	445	1.18	3.97	45	4.48	0.86	4.29

3.2.4 Hydroponic substrates

Three different media were used: Umgeni sand, 6.2 mm gravel and horticultural grade perlite (Figure 3.5). The sand and gravel were sterilised with dilute formaldehyde (30%), rinsed

thoroughly and poured immediately into the pots and troughs. The containers (pots and troughs) were washed with Jik ® (sodium hypochlorite) at a dilution rate of 5 ml Jik ® per 5 L water, and well rinsed with clean water.

Figure 3.5 Pot system showing three different substrates

3.2.5 Experimental design and layout

The trial was established as a modified split-plot design. Substrates were randomised as whole plots and clones as subplots.

3.2.5.1 Pot unit

The block contained three randomised whole plots: gravel, sand and perlite. Each whole plot comprised three clones with 12 ramets/clone randomised throughout the unit. Total number of subplots equalled 36 (Appendix C1.1).

3.2.5.2 Trough unit

The block contained four randomised whole plots: gravel, sand, perlite and NFT (insert). Three of the whole plots (gravel, sand and perlite) contained three clones (subplots) with five ramets/clone randomised within each plot. The NFT plot comprised three clones with seven ramets randomised throughout the whole plot. Total number of subplots equalled 66 (Appendix C1.2).

3.2.6 Preliminary measurements

Prior to the planting of ramets, a series of preliminary tests were conducted to determine the following:

- Temperature of the substrate in individual pots (36) and troughs (4);
- Temperature at the point of discharge and catchment;
- Ambient temperature measurement - both maximum and minimum;
- Flow rate of water to the individual pots (36) and troughs (4);
- Initial electrical conductivity (EC), at point of discharge and leachate from the treatment substrates prior to the introduction of plant material; and
- Initial pH at point of discharge, pH of the leachate and pH at the return trough to tank.

The information obtained from the above assisted in adjusting settings and reducing unnecessary bias that may have confounded the results. The measurements are discussed in the results.

3.2.7 Temperature data

The ambient temperatures were measured daily using a minimum/maximum thermometer attached at the apex of each unit. Pot media temperatures were measured using research quality mercury thermometers. Three 'dry' pots of each media type were randomly selected from each treatment and temperatures recorded after a ten minute period. The pots were then irrigated and left to drain for ten minutes. These readings were recorded as 'wet' temperatures. Trough temperatures were measured in the same fashion at three measured points along the length of each treatment: the inlet pipe, the centre point and the outlet pipe. 'Wet' and 'dry' measurements were also recorded for this system. Pot and trough measurements were recorded three times per day over several days.

3.2.8 Flow rates

The unadjusted flow rate was determined using a stop watch and a measuring cylinder. Once all flow rates were measured for individual nozzles, the flow rates were adjusted to as close

as possible to equal output per nozzle. Flow rate was determined using the following equation:

flow rate = volume of water collected (V_{Tx})
time to collect water (T_x)

3.2.9 Volume and infiltration rates

Infiltration rates for the pot system were determined by measuring the height of the pot and recording the time taken for nutrients to flow from the nozzle to the appearance of leachate at the base. Three readings from each substrate type were recorded in order to calculate a mean. The volume of the frustrum of a right circular cone was calculated using the following equation (BREDENKAMP *et al.*, 1984):

Volume = (1/3) ¶ h ($r_1^2 + r_1 r_2 + r_2^2$)

Where h = height

r_1 = radius of top

r_2 = radius of base

3.2.10 Nutrient solution

The commercially available fertiliser Hydroponica® and calcium nitrate were utilised as the 'control' nutrient. Different ratios of Hydroponica® and calcium nitrate were mixed with water to determine solution pH and electrical conductivity (EC) concentrations. These ratios were used to extrapolate the quantities of nutrients required for addition to different volumes of water.

3.2.11 pH and electrical conductivity (EC) measurements

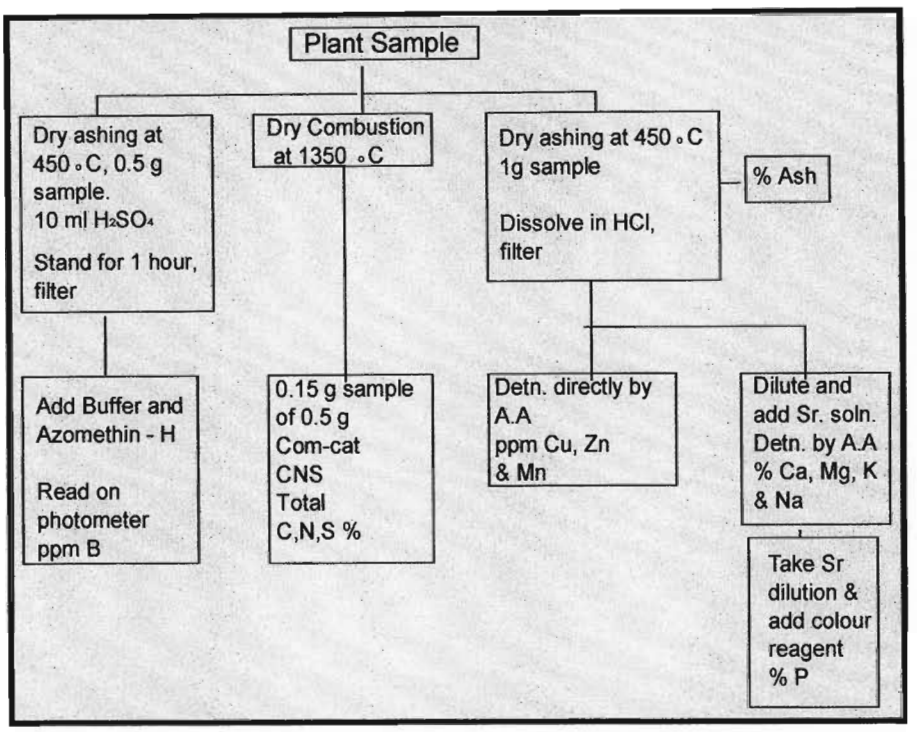
Samples of water in the supply tank (static and circulating), discharge from the outlet of each system and leachate from three pots of each media were sampled. The temperature, EC and pH measurements of each of the samples were recorded using a mercury thermometer, EC meter and pH meter. Readings were recorded for undiluted tap water and after the addition of varying masses of control fertilisers (**x** g $^{-1}$ Hydroponica® + **x** g $^{-1}$ calcium nitrate + 90 L water).

3.2.12 Plant tissue analysis

Nutrient analyses were carried out by the Cedara Plant Laboratory. Samples were dried at 75 °C for 24 hours and milled. Dry samples were ground to pass through a 40-mesh screen fitted

to a rotary mill. Results were reported on a 100% dry-matter basis. The analysis technique for the determination different of nutrient concentrations is summarised in Figure 3.6.

Figure 3.6 Summary of nutrient analysis technique utilised by Cedara Plant Laboratory



(RIEKERT *et al.*, 1998)

3.2.13 Stem cuttings

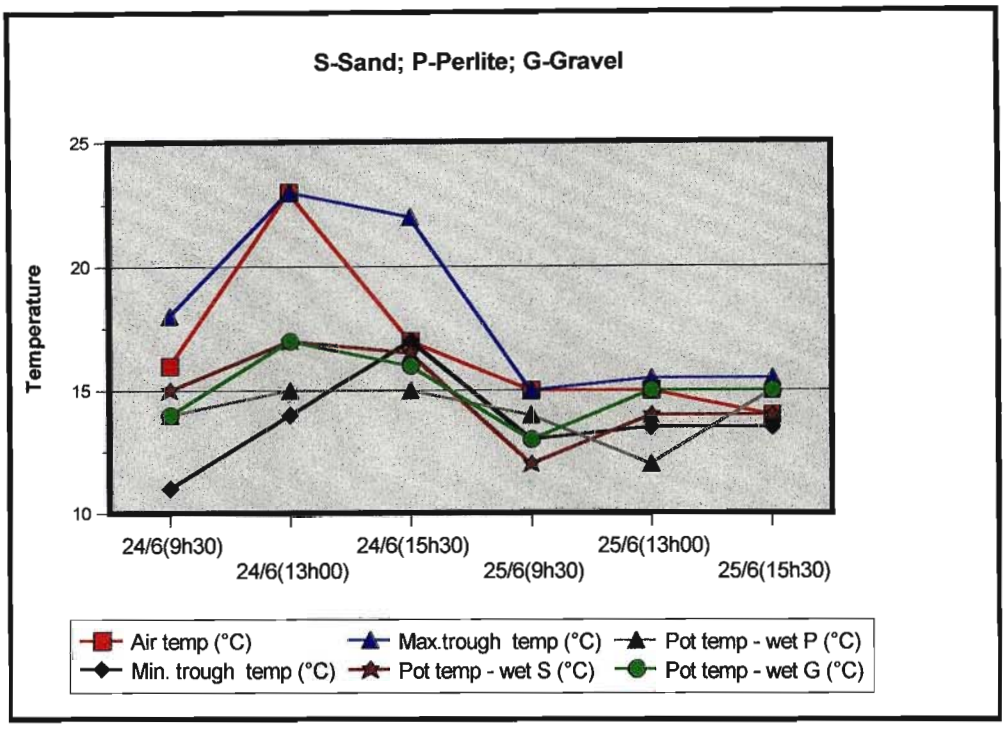
Stem cuttings were prepared from the juvenile shoots of hydroponic mother plants. Cuttings were approximately 5 cm in length and 1.5 to 2.0 mm in diameter. Two leaves per cutting were cut back to 50% of the leaf surface area to prevent overlapping of laminae. Cuttings were immersed for 30 seconds in a benomyl fungicide solution (2 g/L). After immersion, the cuttings were dipped in Seradix No. 3 (4-Indol-3-yl-butyric Acid) (IBA) hormone powder (a.i. 8 mg/kg) and set to a depth of 1 cm in 'Unigro' inserts of 60 mL volume. The media mixture comprised 70% vermiculite, 30% perlite and 10% coconut coir. Prepared trays of cuttings were placed in a greenhouse with bottom heating at 30 °C and misted for 12 seconds every 30 minutes. Day temperature ranges were 20-30 °C and relative humidity was in excess of 75%. No supplementary lighting was provided and cuttings were left to root for 40 days in the tunnel prior to their removal. Rooting assessment was done 60 days later.

3.3 RESULTS AND DISCUSSION

3.3.1 Temperature

The effect of ambient temperature on saturated pot and trough temperatures is shown in Figure 3.7. The dry values are discussed in the results.

Figure 3.7 Temperature fluctuations for different substrates - pot and trough system



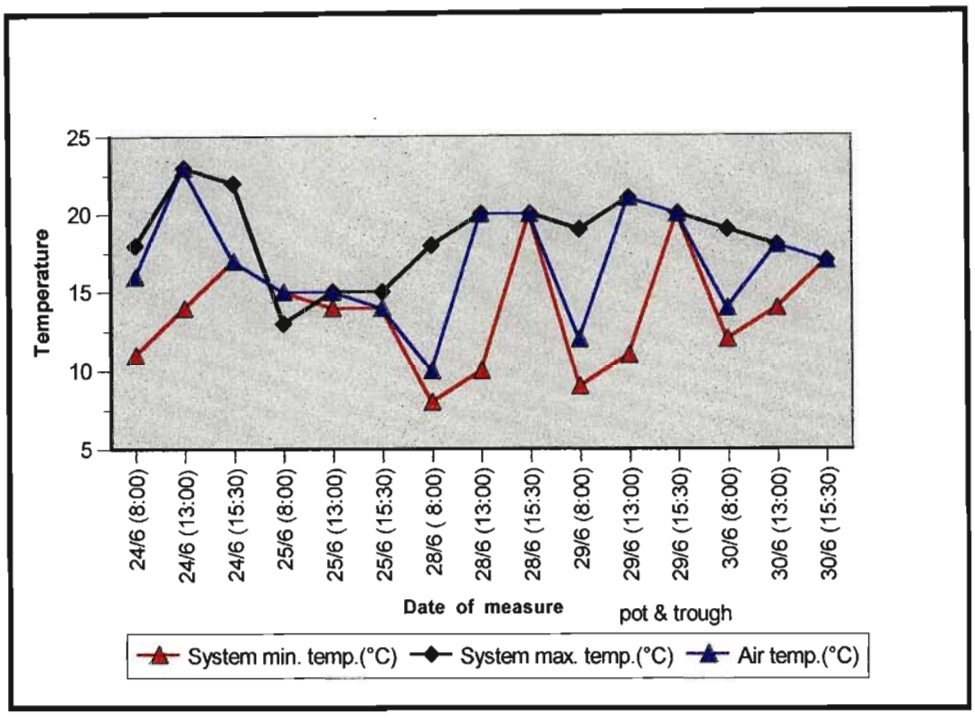
Linear regressions were calculated to establish the degree of linear dependence (assumed) of the substrate temperatures at saturation (wet) and after draining for 12 hours (dry), on ambient temperature. The relevant data is summarised in Table 3.3. All correlations between ambient temperature (explanatory variate) and substrate temperatures (response variates) were relatively strong (>0.7) and the model showed a linear relationship. The best fit was for dry gravel and this model accounted for 73.6 % of the variance (high).

Table 3.3 Correlation values for linear regression calculated for ambient temperature versus substrate temperatures, dry and wet (d.f.=13; ambient mean temp =16.80 °C)

Substrate	s.e.	*r*- value	F. pr.	Mean temp °C	% variance
Gravel wet	1.51	0.830	<0.001	14.13	65.8
Gravel dry	1.88	0.870	<0.001	14.43	73.6
Perlite wet	1.26	0.760	0.003	13.43	54.1
Perlite dry	1.98	0.720	0.001	13.33	47.9
Sand wet	1.54	0.760	0.003	14.03	54.3
Sand dry	2.45	0.710	0.003	13.80	46.9

To test whether substrate estimates differed significantly from their dry to wet states, a series of t-tests (paired data) were calculated. There was no significant difference for any of the substrates when comparing the wet state to the dry state with p-values ($t_{d.f.14}$) ranging from 0.432 for gravel to 0.757 for perlite. There was a strong linear dependence of substrate temperature linked to ambient temperature, but no significant temperature differences between the wet and dry states of the media.

Figure 3.8 Temperature relationships: ambient versus system mean temperature for both pot and trough (measured 24/6/99 to 30/6/99)



Simple linear regressions were calculated using Genstat 5 (release 3.2). The full analysis is presented in Appendix C. The first regression of ambient temperature and its effect on the maximum trough temperature (Figure 3.8) revealed a weak correlation (r = 0.149). The F-value was not significant (F. pr. 0.530) and the straight line fit was poor with data scatter following a more random pattern. The regression of ambient temperatures and the trough minimum response temperature revealed a moderate to strong correlation (r = 0.692). The F-value was significant at the 1% level (F. Pr. <.001) and suggests a linear response of minimum trough temperatures to ambient temperature changes. Maximum pot temperatures had a very weak correlation to ambient temperature (r = 0.288) and the F-value was not significant (F. pr. = 0.218). The regression of ambient temperatures and the pot minimum response temperature revealed a moderate correlation (r = 0.633). The F-value was significant (F. pr. 0.003) and suggests a moderate linear response of minimum trough

temperatures to ambient temperature changes. The response of system temperatures measured in the substrate (pooled data) indicated that ambient temperature had a greater effect in cooling the root zone temperature than in warming it during the warmest part of the day. For every 1 °C drop in ambient temperature the extrapolated trough system temperature (measured in the media) dropped 0.66 °C and in the pot system dropped 0.64 °C. This trend may be reversed in winter, but the regression results indicate that low ambient temperatures will have a much greater effect on decreasing media temperatures than on warming them. This could have deleterious effects on root zone temperatures and must be taken into account with the onset of winter. It may be advisable to consider heating the nutrient solution using submersible type tank heaters to maintain the reservoir solution at 25 °C.

3.3.2 Flow rate

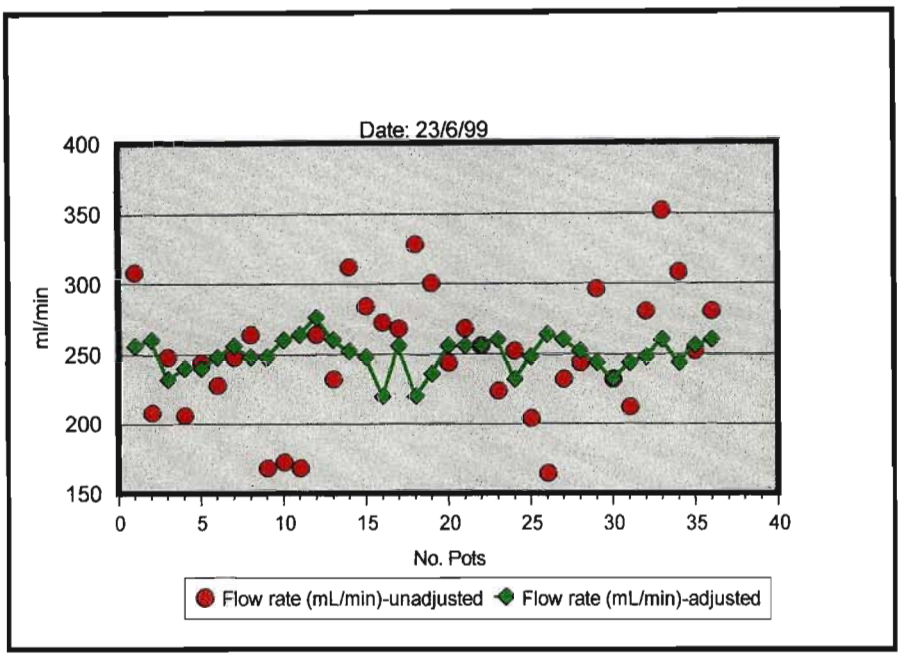
To ensure parity and reduce experimental bias, all nutrient flow rates had to be as similar as possible. Initial rates (unadjusted flow rates) relying on the valve cock settings showed major volume differences. All 36 flow valves had to be individually set to achieve a more accurate output. (Figure 3.9). This is an area requiring major attention, especially where a recirculating pot system is selected. Flow rates that are too high can result in over-saturation of the substrate and the development of anaerobic conditions leading to high stress levels in the root plug, root sloughing and die-back. *Pythium* sp. tends to also thrive in such conditions. The flow rate required by gravel is much higher than that sustained by sand. Table 3.4 summarises statistical values for the unadjusted and adjusted flow rates.

Table 3.4 Summary statistics for unadjusted and adjusted flow rates

	Unadjusted flow rate	Adjusted flow rate
Mean	250.61	249.78
Median	250.00	252.00
Minimum	164.00	220.00
Maximum	352.00	276.00
Range	188.00	56.00
Variance	2074.93	152.18
Standard deviation	45.55	12.34
Standard error	7.59	2.06
Coefficient of variation	18.18	4.94
Skewness	-0.06	-0.26

The sample data showed signs of skewed distribution (Table 3.4) and a t-test (paired) was therefore an unsuitable statistic method to determine differences between data sets. From Table 3.4 it is evident that the **adjusted** flow rates were equalised to a greater point of accuracy. The standard deviation around the mean reduced from 45.55 to 12.34 mL/min and the standard error reduced from 7.59 to 2.06. The range of flow rates reduced from 188.00 to 56.00 mL/min.

Figure 3.9 Distribution of flow rate for recirculating pot system (unadjusted and adjusted flow rates)



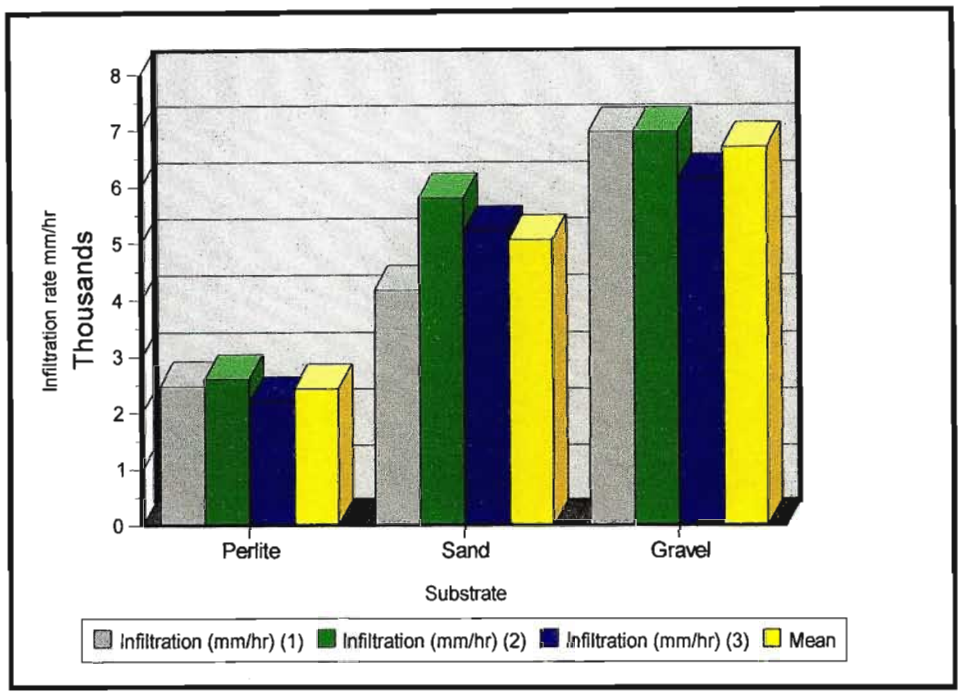
3.3.3 Volume and infiltration rates

HANDRECK *et al.* (1994) note that infiltration rate is measured as mm (height) of water soaking in or through per hour. It can vary from zero for water repellent sands to hundreds of mm per hour for coarse sand.

The infiltration rate of any medium decreases as it becomes wetter finally reaching a steady state value that is maintained until the drainage system clogs up. It is this steady infiltration rate that is most important. The need to increase infiltration rates is much more common than the need to decrease it. Certain potting media allow water to run through too quickly and this has a major impact on the air-filled porosity of the substrate. Immediately after drainage has stopped, the medium at the bottom of the container remains saturated unless it is sitting on an absorbent surface. The actual proportions of water and air in the pore space of the media depends on the size of the pores. The height of saturated and very wet medium is the same

no matter what the height of the container. This means that a medium in a shallow container will have a high average water content and a lower air-filled porosity than the same medium in taller containers. Media used for such containers must have a higher proportion of large pores if the roots in pots are to get enough oxygen (HANDRECK *et al.*, 1994).

Figure 3.10 Infiltration rate for different test substrates



A one-way ANOVA (no blocking) was calculated to determine whether differences existed between substrates. The degrees of freedom (d.f.=8) were too low for the data set to give an accurate indication of the differences and not enough replications existed as a result of the design limitations, but the F-value indicated that there were significant differences between substrates (F. pr. <0.001). This is also very apparent from Figure 3.10. The range in values was 4738.72 mm/hr, with a minimum value of 2221.28 mm/hr (perlite) to a maximum value of 6960.72 mm/hr (gravel). From an effective drainage perspective, it should be noted that although gravel allowed for rapid infiltration, it dried out too quickly and the air pores tended to be too large. A coarser grade of sand than used in this experiment would have been preferable with a target infiltration rate of 3000 mm/hr.

3.3.4 Nutrient solution

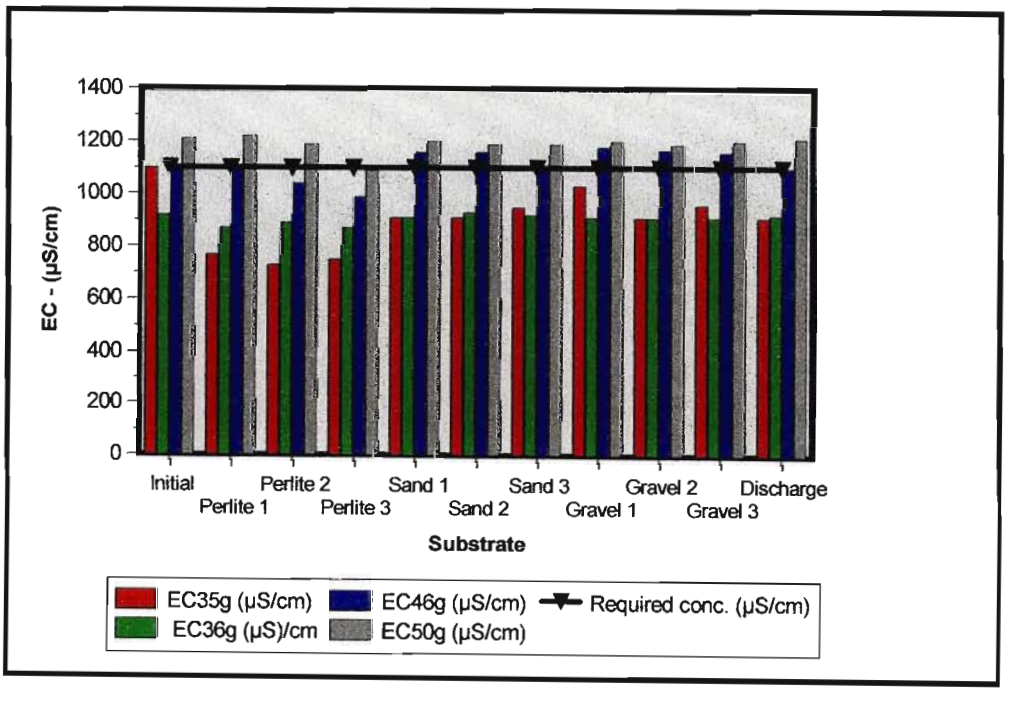
3.3.4.1 Determining fertiliser concentrations

Based on agricultural crop norms for hydroponic systems, an EC level of 1100-1200 µS/cm was selected as the control concentration. Different ratios of Hydroponica® and calcium nitrate ($Ca(NO_3)_2$ were dissolved in warm water and the pH and EC recorded. The results were used to determine the mass of calcium nitrate ($Ca(NO_3)_2$ and Hydroponica® required for the tank mixture (Figure 3.11). The pH of the solution was not adjusted.

The following ratios were tested and EC levels measured:

- 10 g of Hydroponica® + 10 g of calcium nitrate ($Ca(NO_3)_2$ + 90 L of water - EC level too low.
- 35 g of Hydroponica® + 35 g of calcium nitrate ($Ca(NO_3)_2$ + 90 L of water - EC level too low.
- 36 g of Hydroponica® + 36 g of calcium nitrate ($Ca(NO_3)_2$ + 90 L of water - EC level too low.
- 46 g of Hydroponica® + 46 g of calcium nitrate ($Ca(NO_3)_2$ + 90 L of water - EC level acceptable range (1100-1200 µS/cm).
- 50 g of Hydroponica® + 50 g of calcium nitrate ($Ca(NO_3)_2$ + 90 L of water - EC level acceptable range (1100-1200 µS/cm).

Figure 3.11 Electrical conductivity values. Comparison at different masses of control fertiliser, Hydroponica® and calcium nitrate (no pH adjustment)



3.3.4.2 The effect of pH on electroconductivity (EC)

Once the optimum fertiliser levels to achieve an acceptable EC (1100-1200 µS/cm) had been determined, the modifying effect of the addition of acid (30% strength sulphuric acid) to the

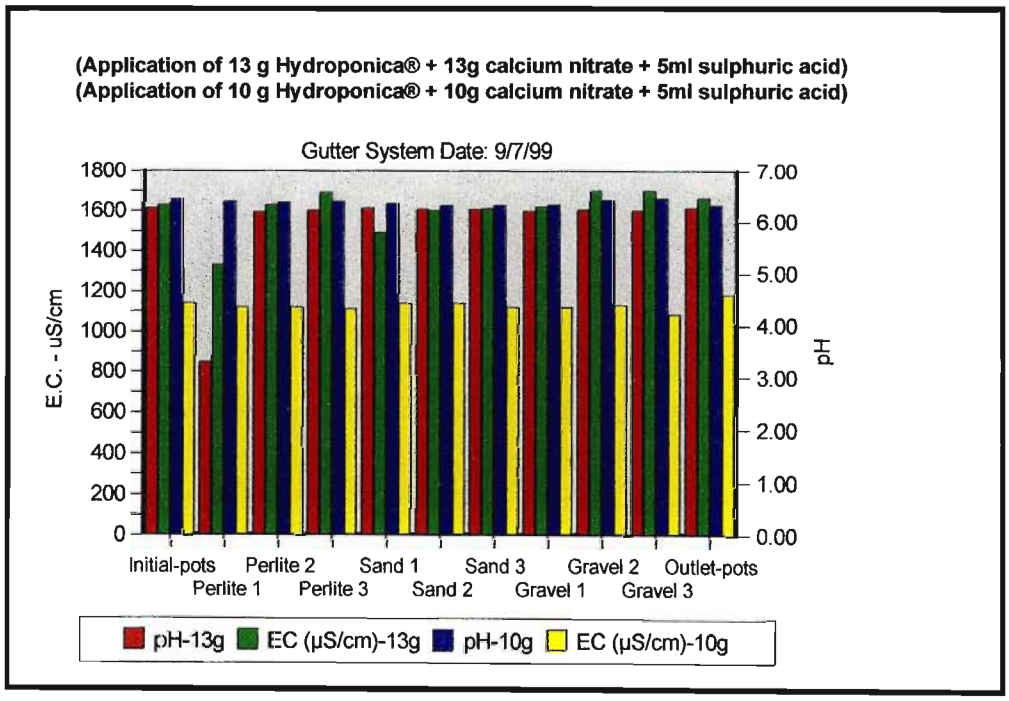
solution concentration was examined. A volume of 5 mL of sulphuric acid was added to two different concentrations of fertiliser solution. Readings were recorded for the following combinations:

- Control - Umgeni water - 90 L volume.
- 10 g Hydroponica® + 10 g calcium nitrate ($Ca(NO_3)_2$ + 5 mL H_2SO_4 + 90 L water.
- 13 g Hydroponica® + 13 g calcium nitrate ($Ca(NO_3)_2$ + 5 mL H_2SO_4 + 90 L water.

Temperature, EC and pH readings of samples of the solution from the supply tanks (static and flowing), discharge from the systems and leachate from three pots of each substrate were recorded (Figure 3.12).

From the previous test (Figure 3.11), a mass of up to 46 g of each fertiliser was required to reach the EC level of 1100 -1200 µS/cm. Through the addition of 5 mL sulphuric acid to pure water, the mass of fertiliser required decreased 3.54 fold. The desired EC range of 1100 -1200 µS/cm was achieved through the addition of 10 g of Hydroponica® and 10 g ($Ca(NO_3)_2$, whilst at 13 g of each nutrient, the EC rose to between 1350 - 1650 µS/cm. Hence, the addition of sulphuric acid to water prior to dissolving of fertilisers can greatly enhance the availability of nutrients in terms of concentration and thus reduce the overall quantity of fertiliser required.

Figure 3.12 Electrical conductivity and pH changes through addition of sulphuric acid (5 mL acid @ 30% volume)



A simple linear regression calculation shows that the r^2 value (squared coefficient of correlation or coefficient of determination) was much stronger at a higher fertiliser mass, r^2 = 0.693 (13 g) vs. r^2 = 0.265 (10 g) (Figure 3.12). At higher masses of fertiliser the relationship between pH (explanatory variable) and EC (response variable) was much more linear and the correlation coefficient, r = 0.832, indicated a strong measure of intensity of association between the two variables. The r^2 value (r^2 = 0.693) showed how much variability was accounted for by regressing EC on pH values; i.e. it represents the measure of the strength of the straight line relationship.

3.3.4.3 The effect of temperature on pH

Prior to the above series of measurements, no baseline data existed from which to extrapolate the effect of temperature on pH (Figure 3.13). This relationship (if it does exist) has serious implications for winter crops. It was hypothesised that temperature does effect pH and that a fluctuating temperature could result in changing pH and EC levels. The temperature and pH were recorded in the varying substrates (sand, perlite and gravel) for undiluted tap water (EC = 80 µS/cm). A series of simple linear and multiple linear regression models (Genstat 5, version 3.2) were determined to establish the significance of relationships. Utilising temperature as the explanatory variate and EC as the response variate (d.f. = 12), the calculated F-value (F. pr. 0.563) indicated no significance in the linear dependence. Utilising temperature as the explanatory variate and pH as the response variate, the calculated F-value (F. pr. 0.573) still indicated no significance in the linear dependence, showing that for the given data set in winter, there was no linear response for either EC or pH to temperature fluctuations.

A simple linear regression calculation using EC as the response variate and pH as the explanatory variate determined a strong significance value (F. pr. <0.001) and the model accounted for 71.7% of the variance. There was a strong correlation (r = 0.861) between EC and pH, and hence a dependency relationship can be predicted.

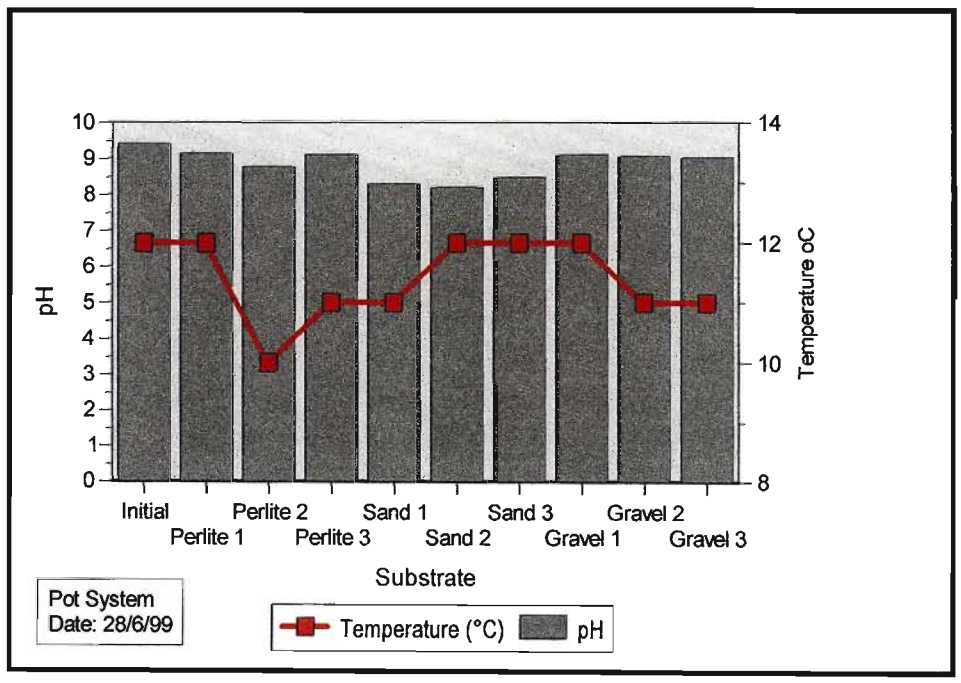
Utilising multiple linear regression (no groups) with the response variate EC and the fitted terms (explanatory variates) pH and temperature, there was a strong significance (F. pr. <0.001) and the model accounted for 82.0 % of the variance.

The correlation matrix revealed the following findings:

pH	**1**	1.000		
EC	**2**	**0.861**	1.000	
Temp	**3**	0.173	-0.018	1.000
		1	**2**	**3**

The strong correlation (r = 0.861) between EC and pH revealed a linear dependence. However, temperature did not correlate with pH (r = 0.173) nor did it correlate with EC (r = -0.018). It can therefore be deduced that during winter months, temperature had no linear relationship to either pH or EC. However, the relationship between pH and EC was very strong. There may be a different response during summer months or under less controlled environmental conditions, but this cannot be extrapolated from these findings.

Figure 3.13 Relationship between temperature and pH (undiluted water EC = 80 µS/cm)



3.3.5 Plant tissue analysis

Prior to attempting to establish optimum nutrient levels in eucalypt hydroponic hedge plants, conventional field clones were analysed to aid in identifying the existing base line. Early results from data are shown in Table 3.5.

Table 3.5 Plant tissue analysis from field hedge plants (South African and Brazilian data)

ID No.	N %	P %	K %	Ca %	Mg %	Na %	B mg/kg	Zn mg/kg	Cu mg/kg	Fe mg/kg	Mn mg/kg	S mg/kg	C %
NH000	2.54	0.22	1.47	0.62	0.19	0.08	*	39	12	*	704	*	46.7
GN107	2.61	0.27	1.47	0.58	0.17	0.12	*	41	14	*	784	*	47.2
GN156	2.52	0.24	1.51	0.51	0.19	0.11	*	43	14	*	538	*	47.2
E.GxN mean	2.65	0.3	1.08	0.77	0.24	0.23	29	44	12	*	1139	*	*
Brazilian range **	2.5 -3.0	0.2-0.4	1.5-2.0	1.0-1.5	0.25-0.4	>.005	40-70	50-60	10-15	100-200	100-500	0.15-0.25	*
SA range	1.40-2.20	0.2-0.4	0.4-1.5	0.2-0.4	0.1-0.2	*	*	30-150	20-40	*	100-250	*	*

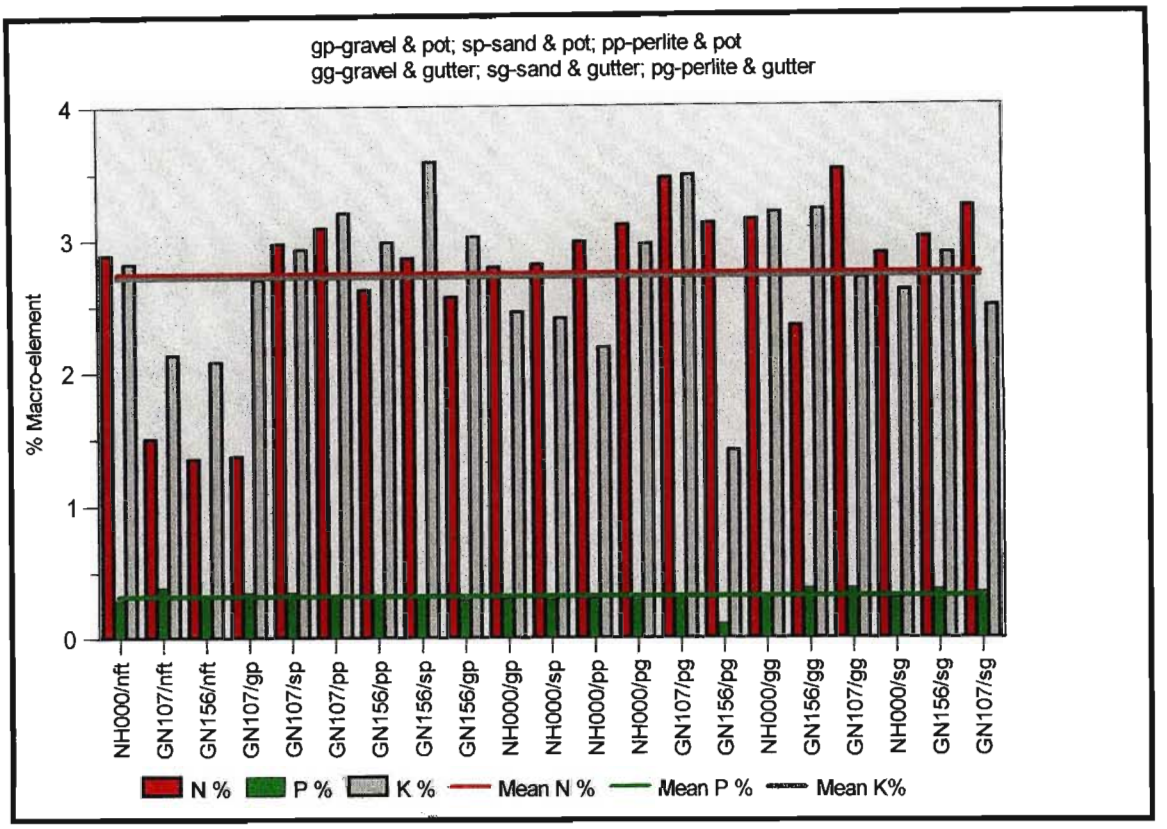
* Data values unknown, ** Brazilian Data (DE ASSIS, 2001), South African data based on mean of 16 sets of measurements. Mean moisture content = 68.73%.

On 11/1/2000 the first set of E.GxN hybrid material was submitted to Cedara Plant Laboratory for dry mass analysis (Figure 3.14). The objective was to establish a baseline from which to determine the individual element levels (macro- and micro-elements) for future experimental work. The summary statistics across all systems, clones and substrates are shown in Table 3.6.

Table 3.6 Summary statistics for primary elements from first hydroponic planting

Summary statistic	N %	P%	K%
Mean	2.74	0.32	2.73
Median	2.89	0.33	2.82
Minimum	1.35	0.11	1.41
Maximum	3.52	0.39	3.58
Range	2.17	0.28	2.17
Standard deviation	0.62	0.06	0.52
Standard error of mean	0.14	0.01	0.11
Coefficient of variation (CV)	22.69	18.19	18.87
Skewness	-1.29	-2.15	-0.63

Figure 3.14 Primary macro-element concentration of test clones at establishment



A statistical summary of the baseline data for the secondary macro-elements is indicated in Table 3.7).

Table 3.7 Statistical summary of secondary elements from first hydroponic planting

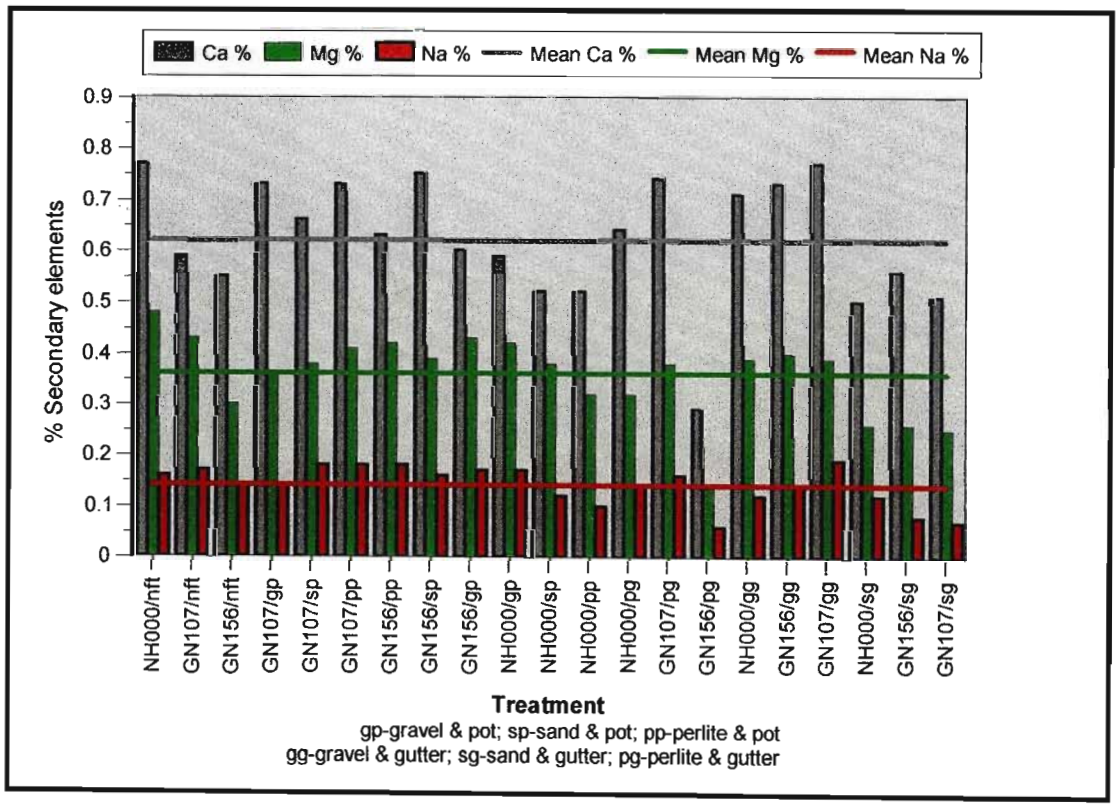
Summary statistic	Ca %	Mg %	Na %
Mean	0.62	0.36	0.14
Median	0.63	0.38	0.14
Minimum	0.29	0.14	0.06
Maximum	0.77	0.48	0.19
Range	0.48	0.34	0.13
Standard deviation	0.12	0.00	0.04
Standard error of mean	0.03	0.02	0.00
Coefficient of variation (CV)	19.27	22.25	27.06
Skewness	-0.86	-1.05	-0.73

From an analysis of variance (one-way ANOVA, no blocking) for macro-element differences within clones, there were no significant differences (F. pr. values ranging from 0.370 to 0.657; d.f. = 20) and clone type could be eliminated as a source of variance.

Elements showed no significant variation at the substrate level (F. pr. values ranging from 0.212 to 0.438; d.f. = 20) and substrate could also be eliminated as a source of variance. Differences between elements and system showed a weak significance for magnesium (F. pr. 0.017) and sodium (F. pr. 012). For both elements, the lowest mean values were in the trough system. There was no significant difference for calcium (F. pr. 0.631).

In summary, neither **clone type, substrate** nor **system** showed any significant differences for the secondary macro-element at the 1% and 5% level and all three can be rejected as sources of variance (Figure 3.15).

Figure 3.15 Secondary macro-element concentration of test clones at establishment



It is suggested that micro-elements function as cofactors for many of the plant growth regulatory processes and that they may affect rooting to a greater extent than the macro-elements. A statistical summary of the baseline data for the micro-elements at establishment is indicated in Table 3.8.

Table 3.8 Statistical summary of micro-elements from first hydroponic planting

Summary statistic	B mg/kg	Cu mg/kg	Mn mg/kg	Zn mg/kg
Mean	257.67	34.05	348.52	232.48
Median	227.00	33.00	309.00	148.00
Minimum	174.00	25.00	174.00	76.00
Maximum	398.00	46.00	621.00	622.00
Range	224.00	21.00	447.00	546.00
Standard deviation	60.59	5.29	135.15	176.53
Standard error of mean	13.22	1.15	29.49	38.52
Coefficient of variation (CV%)	23.52	15.53	38.78	75.93
Skewness	0.56	0.34	0.62	0.98

From Table 3.8 it is evident that the data for the micro-elements is positively skewed (not normally distributed) as compared to the negative skew for the macro-elements.

Analysis of variance (one-way ANOVA, no blocking) for differences between test clones for micro-elements showed no significant differences for boron, manganese and zinc (F. pr. values ranging from 0.376 to 0.915; d.f. = 20), but copper showed some significance, although weak, (F. pr. 0.009) and this can be accounted for by clone NH000 with a mean of 29.5 mg/kg. It could be safely assumed that at the 1% and 5 % levels, clone type could be eliminated as a source of micro-element variance.

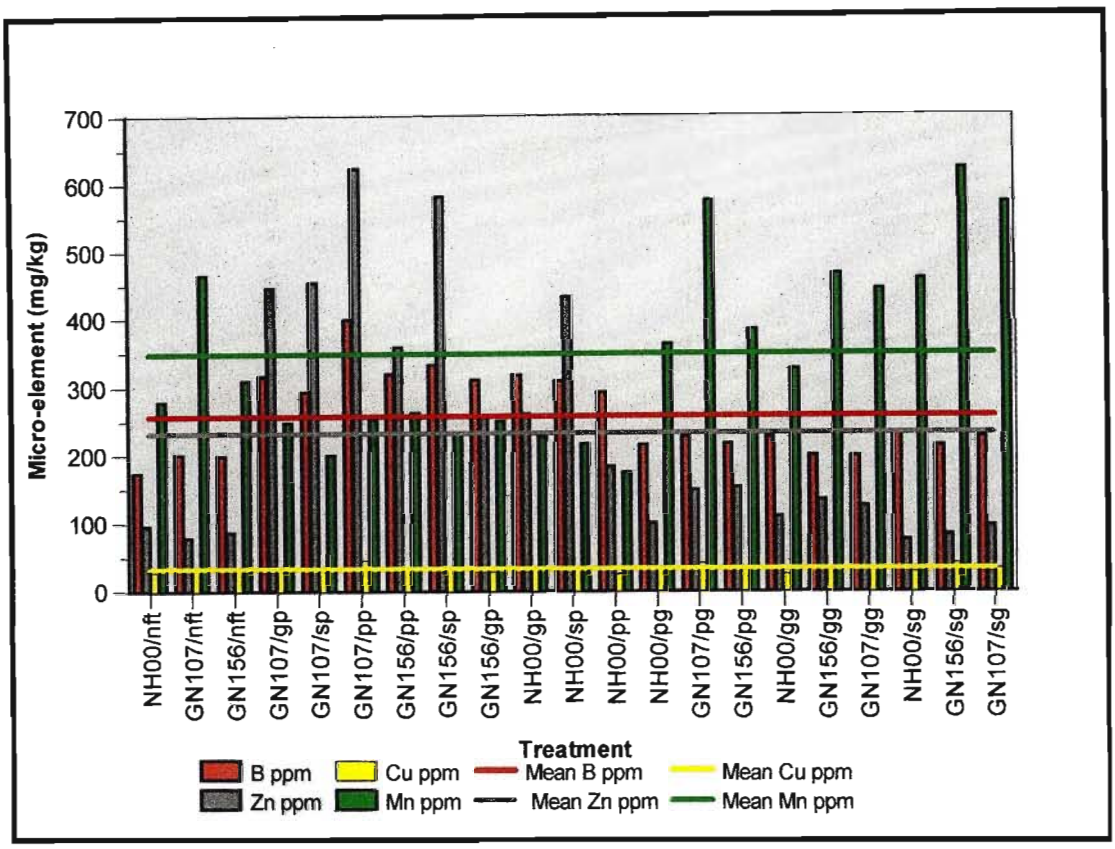
Differences between element concentration and varying substrates showed no significance (F. pr. values ranging from 0.224 to 0.909; d.f. = 20) and substrate could also be eliminated as a source of variance.

Differences between hydroponic systems for micro-element concentrations produced an interesting output. There were significant differences between systems for boron, manganese and zinc (F. pr. <0.001). For the trough system, boron and zinc had lower mean values, whilst for manganese the pot system had a lower mean concentration. There was no significant difference for copper (F. pr. 0.841).

In summary, neither **clone type** nor **substrate** showed any significant differences for the micro-element at either the 1% or 5% level. However, **system** differences were apparent for three micro-elements, boron, manganese and zinc. The reason for the strong effect of the

different systems on micro-element concentration (Figure 3.16) could not be explained in the context of this experiment.

Figure 3.16 Micro-element concentrations of test clones at establishment



3.3.6 Experimental rooting results

On 13/3/2000 a count of rooted cuttings from the hydroponic units was completed and the results are summarised in Table 3.9. Comparisons with the field control proved to be difficult due to the disparate numbers of cuttings generated from the different hedge types and the obvious age differences. The mean **field hedge** rooting across all clones was 50.88% whilst the mean rooting of clones from the **hydroponic unit** was 48.57%. The data sets were too small for accurate comparisons, but t-test results (unpaired) showed no significant difference. Henceforth, all comparisons were carried out only between the two hydroponic units.

Rooting percentages are often misleading in describing the efficiency of a clone's performance. Theoretically, one clone may produce very low numbers of cuttings that root at high levels (>70%) whilst another commercial selection will produce a large quantity of coppice material that roots at mediocre levels (50 - 60%). The confounding factor of such results leads to difficulties in determining the optimum number of hedge plants to establish in order to produce the budgeted number of rooted plants. An alternative is to express a derived

(calculated on rooting potential) number of cuttings to be set to produce 100 rooted plants (Table 3.9). The lower the derived number set to produce the 100 plants, the better the rooting efficiency, i.e. if a selected hybrid clone has a consistent rooting of 75%, 133 cuttings would have to be set to produce the required 100 plants (Figure 3.17). However, for a clone with a known rooting of 35%, it would be necessary to set 286 cuttings to achieve the rooting of 100 plants. Such information could assist nurserymen in determining the size of their clonal banks, be they conventional field hedges or 'hydroponic gardens'.

Table 3.9 Rooting results for recirculating hydroponic systems as at 13/3/2000, expressed as a derived number of cuttings set to root 100 plants

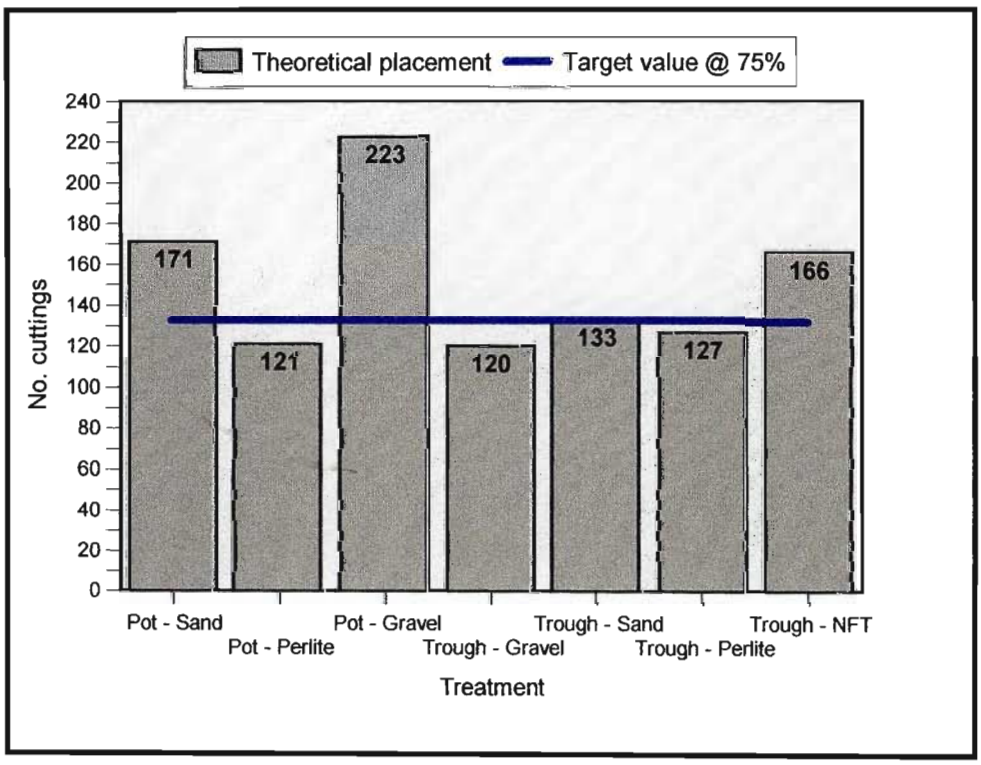
System	Treatment	Clone ID	Cuttings placed	Cuttings Rooted	Rooting %	Harvest Factor	Theoretical placement /100
Pot	Sand	GN156	8	5	62.50	0.625	160
Pot	Sand	GN107	4	2	50.00	0.500	200
Pot	Sand	NH000	0	0	0.00	0	0
Sum			**12**	**7**	**58.33**	**0.583**	**171**
Pot	Perlite	GN107	12	11	91.67	0.917	109
Pot	Perlite	GN156	1	0	0.00	0.000	0
Pot	Perlite	NH000	4	3	75.00	0.750	133
Sum			**17**	**14**	**82.35**	**0.824**	**121**
Pot	Gravel	GN156	13	7	53.85	0.538	186
Pot	Gravel	GN107	13	5	38.46	0.385	260
Pot	Gravel	NH000	3	1	33.33	0.333	300
Sum			**29**	**13**	**44.83**	**0.448**	**223**
Trough	Gravel	GN107	13	11	84.62	0.846	118
Trough	Gravel	NH000	7	6	85.71	0.857	117
Trough	Gravel	GN156	28	23	82.14	0.821	122
Sum			**48**	**40**	**83.33**	**0.833**	**120**
Trough	Sand	GN107	30	21	70.00	0.700	143
Trough	Sand	NH000	17	14	82.35	0.824	121
Trough	Sand	GN156	25	19	76.00	0.760	132
Sum			**72**	**54**	**75.00**	**0.750**	**133**
Trough	Perlite	GN107	17	10	58.82	0.588	170
Trough	Perlite	NH000	33	33	100.00	1.000	100
Trough	Perlite	GN156	26	17	65.38	0.654	153
Sum			**76**	**60**	**78.95**	**0.789**	**127**
Trough	NFT	GN107	38	15	39.47	0.395	253
Trough	NFT	NH000	39	27	69.23	0.692	144
Trough	NFT	GN156	49	34	69.39	0.694	144
Sum			**126**	**76**	**60.32**	**0.603**	**166**
Trough Sum			**322.00**	**230.00**	**71.43**	**0.714**	**140**
Pot Sum			**58.00**	**34.00**	**58.62**	**0.586**	**171**

Analysis of variance (one-way ANOVA, no blocking) to test for the effect of system, substrate and clone on rooting showed that system type had an effect on rooting differences (mean pot

system rooting = 57.8% vs. mean trough rooting = 73.6%) but not at a strong enough test of significance (F. pr. 0.074; d.f.=18). Both substrate (F. pr. 0.497) and clone (F. pr. 0.513) showed no significant rooting differences.

In summary, system type, substrate, and genotype did not significantly affect rooting results. It can be concluded that cultural factors other than these (possibly nutrition and disease) were probably more responsible for the variance.

Figure 3.17 Theoretical placement to produce 100 rooted plants



3.3.7 Relationship of rooting performance to nutrient concentration

On 11/1/2000, harvest material was sent to Cedara Plant Laboratory for dry mass analysis. This data was collated with mean root data for the different test clones and is summarised in Table 3.10 & Table 3.11.

Table 3.10 Plant tissue analysis across systems, substrates and clones (pot unit - 11/01/2000)

System	Clone ID	Substrate	N %	P %	K %	Ca %	Mg %	Na %	B mg/kg	Zn mg/kg	Cu mg/kg	Mn mg/kg	Protein %	Root strike %
Pot	GN107	Gravel	1.37	0.35	2.70	0.73	0.36	0.14	316.00	446.00	37.00	248.00	8.57	38.46
Pot	GN156	Gravel	2.56	0.29	3.02	0.60	0.43	0.17	310.00	259.00	29.00	250.00	15.98	53.85
Pot	NH000	Gravel	2.79	0.30	2.45	0.59	0.42	0.17	317.00	260.00	33.00	227.00	17.43	33.33
Gravel Mean			2.24	0.31	2.72	0.64	0.40	0.16	314.33	321.67	33.00	241.67	13.99	44.83
Pot	GN107	Perlite	3.09	0.34	3.20	0.73	0.41	0.18	398.00	622.00	46.00	253.00	19.30	91.67
Pot	NH000	Perlite	2.98	0.30	2.18	0.52	0.32	0.10	292.00	183.00	25.00	174.00	18.60	25.00
Pot	GN156	Perlite	2.62	0.33	2.98	0.63	0.42	0.18	319.00	358.00	33.00	262.00	16.38	0.00
Perlite Mean			2.90	0.32	2.79	0.63	0.38	0.15	336.33	387.67	34.67	229.67	18.09	70.59
Pot	GN156	Sand	2.86	0.33	3.58	0.75	0.39	0.16	332.00	581.00	37.00	233.00	17.87	62.50
Pot	NH000	Sand	2.81	0.29	2.40	0.52	0.38	0.12	308.00	432.00	33.00	216.00	17.54	0.00
Pot	GN107	Sand	2.97	0.35	2.93	0.66	0.38	0.18	293.00	454.00	37.00	200.00	18.58	50.00
Sand Mean			2.88	0.32	2.97	0.64	0.38	0.15	311.00	489.00	35.67	216.33	18.00	58.33
System Mean			**2.67**	**0.32**	**2.83**	**0.64**	**0.39**	**0.16**	**320.56**	**399.44**	**34.44**	**229.22**	**16.69**	**55.17**

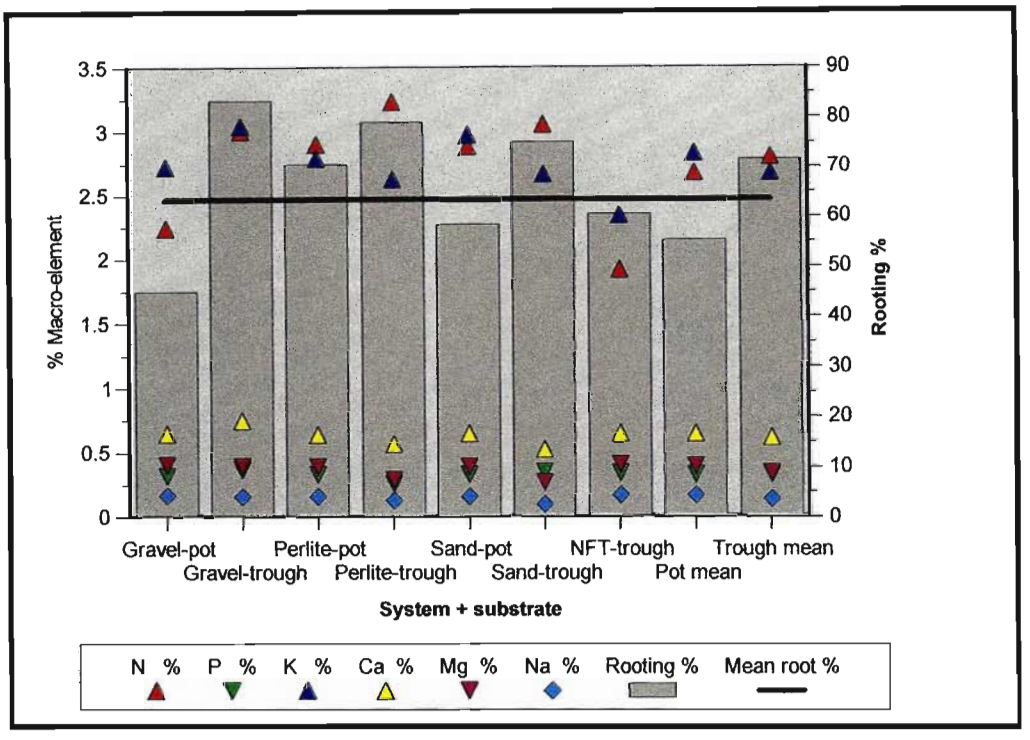
Table 3.11 Plant tissue analysis across systems, substrates and clones (trough unit - 11/01/2000)

System	Clone ID	Substrate	N %	P %	K %	Ca %	Mg %	Na %	B mg/kg	Zn mg/kg	Cu mg/kg	Mn mg/kg	Protein %	Root strike %
Trough	NH000	Gravel	3.15	0.31	3.2	0.71	0.39	0.12	227	110	25	326	19.67	85.7
Trough	GN156	Gravel	2.34	0.38	3.22	0.73	0.4	0.14	199	134	34	466	14.61	82.1
Trough	GN107	Gravel	3.52	0.38	2.7	0.77	0.39	0.19	198	125	33	443	22.02	84.6
Gravel Mean			3.00	0.36	3.04	0.74	0.39	0.15	208.00	123.00	30.67	411.67	18.77	83.33
Trough	GN107	NFT	1.5	0.39	2.13	0.59	0.43	0.17	201	79	33	465	9.35	39.5
Trough	NH000	NFT	2.89	0.29	2.82	0.77	0.48	0.16	174	96	29	279	18.07	69.2
Trough	GN156	NFT	1.35	0.31	2.08	0.55	0.3	0.14	199	87	33	309	8.47	69.4
NFT Mean			1.91	0.33	2.34	0.64	0.40	0.16	191.33	87.33	31.67	351.00	11.96	60.32
Trough	GN107	Perlite	3.46	0.34	3.48	0.74	0.38	0.16	227	148	42	574	21.65	58.8
Trough	NH000	Perlite	3.11	0.29	2.96	0.64	0.32	0.14	214	100	33	362	19.41	100
Trough	GN156	Perlite	3.12	0.11	1.41	0.29	0.14	0.06	216	152	38	383	19.52	65.4
Perlite Mean			3.23	0.25	2.62	0.56	0.28	0.12	219.00	133.33	37.67	439.67	20.19	78.95
Trough	GN107	Sand	3.24	0.35	2.49	0.51	0.25	0.07	227	96	34	570	20.26	70
Trough	GN156	Sand	3.01	0.37	2.89	0.56	0.26	0.08	213	84	42	621	18.81	76
Trough	NH000	Sand	2.89	0.3	2.61	0.5	0.26	0.12	231	76	29	458	18.07	82.3
Sand Mean			3.05	0.34	2.66	0.52	0.26	0.09	223.67	85.33	35.00	549.67	19.05	75.00
System Mean			**2.80**	**0.32**	**2.67**	**0.61**	**0.33**	**0.13**	**210.50**	**107.25**	**33.75**	**438.00**	**17.49**	**71.43**

Prior to the calculation of correlation values, an attempt was made to determine whether visual relationships could be established through the graphical representation of the data. Through this 'correlation' it appeared that levels of nitrogen and potassium lower than 2.0% negatively affected rooting whilst at 2.5-3.0% concentration, mean rooting improved.

Phosphorus apparently had no effect on rooting dynamics and appeared very stable (Figure 3.18). The secondary macro-element group of calcium, magnesium and sodium formed a 'stable' group and did not appear to fluctuate with rooting changes (Figure 3.18).

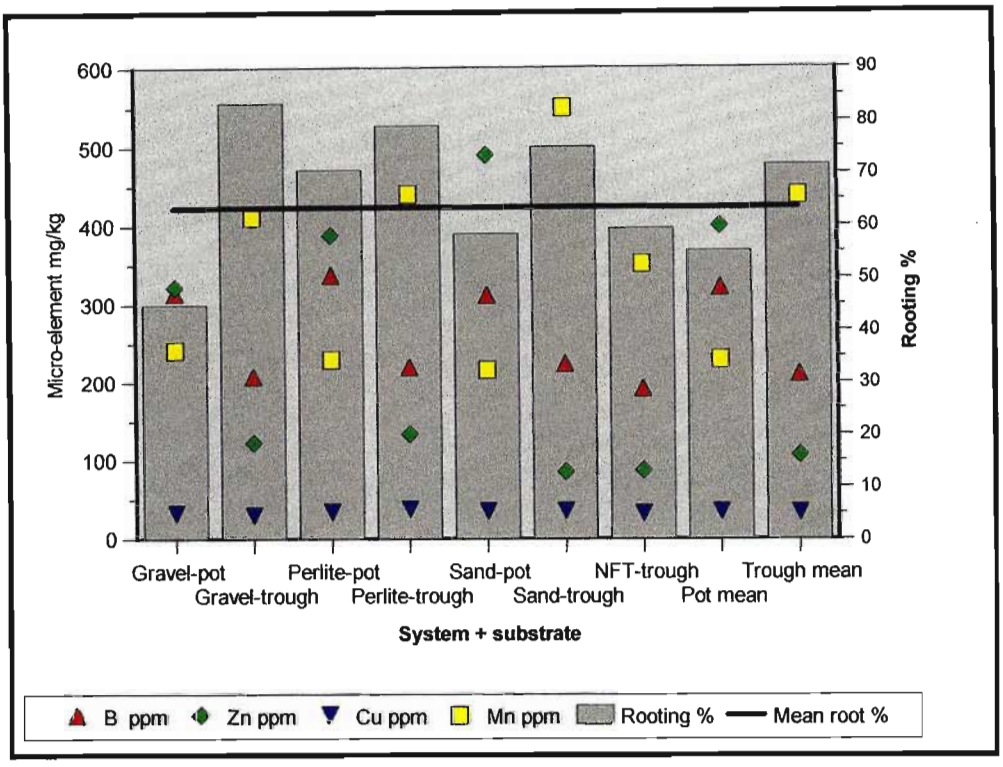
Figure 3.18 Relationship between rooting % and macro-element concentration



Within the micro-element group (Figure 3.19), boron levels of approximately 200 mg/kg had a positive effect on rooting, but beyond this concentration (>300 mg/kg) a toxic response could be assumed to negatively impact on rooting. No visual relationship was apparent for copper and it appeared to be a very stable element. Zinc may have had a beneficial effect on rooting at 100 mg/kg, but at higher concentrations (>300 mg/kg) the rooting response seems negatively correlated. Overall, the response of zinc on rooting appeared generally weak. Optimum rooting linked to manganese concentration was most responsive at the 400-550 mg/kg range, whilst rooting was lowest at the 200-250 mg/kg range.

It is very important that no scientific conclusions be based on these early assumptions from simple visual comparisons. The graphical scales may not clearly indicate individual elemental responses and furthermore, the complexities of elements and their continual interactions and dependencies on one another, can in no way be accounted for by such a simplistic approach. However, it may aid in eliminating the toxic concentration of certain elements and ensure a more balanced ratio of nutrients.

Figure 3.19 Relationship between rooting % and micro-element concentration



3.3.8 Statistical analysis of rooting response to nutrient concentration

McCONWAY and JONES (1999) describe regression in its simplest form as a technique for modelling a linear relationship between two variates, one a response variate and the other an explanatory variate.

A multiple linear regression model was determined for the relationship between rooting and the various nutrients to identify those with the greatest interaction. Utilising all the elements as explanatory variables, the model could only account for a weak 20.1% of the variance and the estimated model for the mean response would be of little or no value.

To develop a more explanatory model for the relationship of rooting and nutrient elements it was necessary to reduce the number of explanatory variables. As clearly indicated by McCONWAY and JONES (1999), we are trying to understand the situation in simplified terms by identifying the main sources of influence on the response variable. A second important point is prediction. Just because we can fit data at our disposal, does not mean that the resulting model will be anything like such a good fit for further data that arises. It is better not to 'over-fit' the current data, by using large numbers of explanatory variables, but to use just enough explanatory variables to capture the main features.

For the data set, the model resulting from step-wise regression contained just two explanatory variables, **calcium** and **magnesium**. The correlation between calcium and magnesium was 0.763 and although not strong (r >0.800), McCONWAY and JONES (1999) suggest that this level of correlation need not seriously preclude the two variables from appearing together.

The best linear equation for the effect of the elements on rooting can be represented as: **rooting % = 41.3 + 220.8 Ca - 335.4 Mg**. The percentage variance accounted for increased from 20.1% for the 10 variable model to 36.9 % for the two-variable model.

3.3.9 Threat from pathogens

Fungal attack by plant pathogens posed a constant threat to the success of the experiment. To reduce hydroponic hedge plant losses, a weekly application of fungicides was introduced. The fungicide Sporegon® was sprayed weekly and did not moderate the EC levels as it contains no ionic salts.

In late December 1999 an outbreak of *Pythium sp.* was experienced. A total of nine plants in the pot system died with the symptoms of the pathogen expressed and another seven died at cutback from related causes. A total of six plants died in the trough system. This was most evident in the sand and perlite substrates and may have been as a result of excess water and a lack of adequate drainage. Evidence of the presence of *Pythium* included the classical sloughing of root hairs, and roots turning a brown colour. Roots were also slimy to the touch. It was thought that more plants would die in the trough system because of their closer proximity to one another, but this did not occur. No reasonable explanation can be offered as to why the outbreak was not as severe in the trough as it was in the pot, other than to speculate that the greater volume of media in the pot (especially the greater depth) held more solution which intensified the *Pythium* spore density.

A continual problem was experienced with powdery mildew. Samples were sent to the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, for identification. Associate Professor Teresa Coutinho stated that *Oidium eucalypti* has been reported on eucalypts and it is presumed to be the same fungus.

3.4 CONCLUSIONS

This experiment was the first one ever attempted in Mondi Forests to systematically identify a number of factors that may have an effect on the success or failure of an intensive hydroponic propagation system. The primary objective was to eliminate the worst performing hydroponic system (trough versus pot).

Temperature measurements revealed a moderate correlation between ambient and substrate temperatures (r >0.7). The best fit was for dry gravel and this model accounted for 73.6% of the variance (high).

The response of system temperatures measured in the substrate (pooled data) indicated that ambient temperature had a greater effect in cooling the root zone temperature during the coolest part of the day than in warming it during the warmest part of the day. This could have deleterious effects on root zone temperatures and must be taken into account with the onset of winter. It may be advisable to consider heating the nutrient solution.

VAPAAVUORI *et al.* (1992) studied the growth and gas exchange characteristics in pine *(Pinus sylvestris)* and spruce (*Picea abies*) seedlings grown in hydroponic culture in the presence of nitrogen (50 mg/L) and transferred at the start of their second growing season to tap water at 5, 8, 12, 16, or 20 ^{0}C (air temperature 18-20 ^{0}C) for three weeks for pines and five weeks for spruce. Root growth of both species was completely inhibited at root temperatures of 5 and 8 ^{0}C, but increased almost exponentially as temperature increased. Shoot growth was maximal at 12 ^{0}C for both pine and spruce. In both species, CO_2 uptake was reduced at low temperatures and appeared to be influenced by the pattern of nitrogen translocation. In pine seedlings, as root temperature increased, an increasing proportion of total nitrogen was retranslocated to the new shoot. In spruce seedlings nitrogen was retranslocated to roots. Differences in retranslocation of nitrogen in the two species were reflected in the amount of soluble protein in the needles, which at the end of the experiment increased with increasing root temperature in pines, but decreased in spruce. Data suggested that for spruce, but not for pine, CO_2 uptake was limited by the amount of Rubisco [ribulose-1,5-bisphosphate carboxylase/oxygenase], an important enzyme in the photosynthetic and photo-respiratory pathways.

Temperature can influence rooting by interfering with nutrient uptake and metabolism, and its control, especially in subtropical climates, must be adjusted for optimum cutting production.

Rooting percentages of mini-cuttings in Brazil decreased in cold winter months. Recent studies have shown that this problem can be solved by supplying additional light (14 h/1000 Lux) and increasing the ambient temperature of the mother plant environment to higher than 20 °C (DE ASSIS, 2001). Considering that nutrient concentration is of prime importance in the rooting process and that nutrient uptake depends on metabolic activity, it can be assumed that both temperature and light contribute to the re-establishment of normal rooting competence (DE ASSIS, 2001).

From a substrate drainage perspective, it was found that although gravel allowed for rapid infiltration, it dried out too quickly and the air pores tended to be too large. The advantages and disadvantages of the test substrates are summarised in Table 3.12.

Table 3.12 Matrix of advantages and disadvantages of test substrates in test units

Advantages/Disadvantages	NFT	Sand	Gravel	Perlite
Resistance to algae contamination	xxx	x	xxx	x
Resistance to plant toppling	xxx	x	xx	x
Management of diseases	xxx	x	xx	x
Resistance to saturation	xxx	x	xx	x
Drainage ability	xxx	x	xxx	x
Water cycle management	xxx	x	xx	x
Resistance to desiccation	x	xxx	xx	xxx
Flexibility of system	xxx	x	xx	x

Legend : xxx - excellent, xx - good, x - poor

The addition of acid to the water prior to dissolving of fertilisers greatly reduced (3.54 fold reduction) the mass of nutrient feed required. At higher masses of fertiliser, the relationship between pH and EC was found to be more linear and the correlation coefficient (r = 0.832) indicated a strong measure of intensity of association. There was no corresponding interaction between temperature and pH for the same data set.

Genotype, substrate and system design did not affect macro-element concentrations. For the micro-elements, nutrient concentrations were not significantly affected by clone and substrate differences. However, there were significant differences between systems for boron, manganese and zinc (F. pr. <0.001).

The different hydroponic designs had a weak effect on rooting (mean pot rooting = 57.8% vs. mean trough rooting =73.6%) but not at a strong enough test of significance (F. pr. 0.074).

Substrate and genotype did not significantly affect rooting and other factors (possibly nutrition, disease, seasonality and plant growth regulators) were responsible for the variance.

Calcium and magnesium appeared to interact with rooting. The correlation between calcium and magnesium was relatively strong (r = 0.763) although a higher value would have been desirable (r >0.800). The regression statistics showed that the visual evaluation discussed earlier had no scientific value and such deductions cannot be made without the support of strong statistical facts. At this stage, one cannot clearly identify which elements interact with rooting. It is most probable that the relationship is a series of complex interacting elemental ratios, rather than just a simple one on one linear dependence.

Fungal attack by plant pathogens posed a constant threat to the success of the experiment and its deleterious effect on hydroponic systems cannot be under-estimated. A major loss of hydroponic hedge plants was caused by too frequent a cycle of fertigation and experience illustrated that the cyclic timers should run for a maximum of 15 minutes on-time, for no more than four to five cycles per day. At higher application rates, roots started to turn brown and die back as a result of insufficient drainage. A weekly spraying programme of fungicides was essential.

Although system type did not have a significant statistical effect on rooting performance, there were a number of practical reasons as to why one would select the trough type unit for commercial application to a eucalypt hydroponic hedge system. The cost of building the pot system was higher than that of the trough unit due to the number of additional valves and irrigation pipe fittings required. The adjustment of pot flow rates was extremely sensitive and had to be constantly reset as a pressure actuating switch was not fitted to the pump and flow rates tended to fluctuate. The pot system was inefficient in terms of plant stocking. Whilst the pot unit could accommodate 10 plants/m^2, the trough system easily accommodated 18 plants/m^2 (extra 80 %) and this figure could have been increased to 33 plants/m^2, a 230 % improvement over the pot system. The ease of management of the trough unit, its greater plant capacity and the simplicity of its design lead to this system being selected as the most practical for further experimental purposes.

CHAPTER 4

RESPONSE OF CLONAL EUCALYPT HEDGE PLANTS TO THREE NUTRIENT SOLUTIONS IN A RECIRCULATING HYDROPONIC SYSTEM

4.1 INTRODUCTION

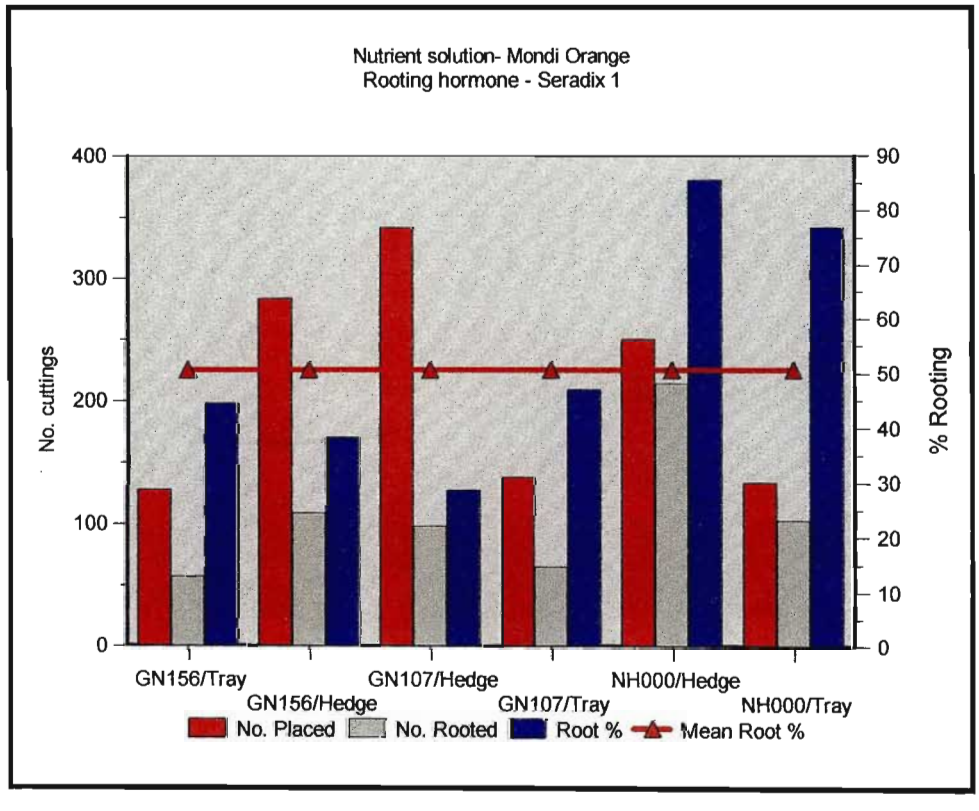
Adventitious root formation can be divided into two stages: 1) dedifferentiation and root initiation and 2) root growth and development (HARTMANN *et al.*, 1990). The first stage is critical for cuttings to root successfully and the significance of mineral nutrient content on root growth and development has been recognised. However, the effect of specific mineral elements on dedifferentiation and root initiation remains unclear. Previous studies provided an understanding of the role of mineral elements in later root growth and development, but mineral elemental effects during the early developmental stages of adventitious root formation are less clear because of the difficulty in separating early endogenous root initiation from visible root growth and development (SVENSON *et al.*, 1995).

Many elements including nitrogen, phosphorus, calcium, magnesium, manganese, boron and zinc are thought to be active in root induction. Zinc, manganese and boron influence the metabolic status of indole-3-acetic acid (IAA). The iron status of plants may be regulated partially by auxins. Despite their importance, the role of micro-nutrient elements on adventitious root formation has received little attention (SVENSON *et al.*, 1995).

Prior to the advent of the hydroponic garden, cuttings were harvested from inserts held in a supporting framework. DE ASSIS *et al.* (1992) and XAVIER and COMÉRIO (1996) noted that the conventional nursery tray provided a series of technical and economical benefits as well as good root quality. However, despite being a great advance over field hedges, mini-hedges (tray hedges) face a number of limitations. The outdoor mini-hedge is still at the mercy of fluctuating weather conditions, and the problems related to adequate nutritional status and leaf disease prevalence continue, especially during winter. The main problems are reduced photosynthetic rates, reduced nutrient uptake and high levels of nutrient loss by leaching during periods of high rainfall. All these limiting factors resulted in the development of the indoor hydroponic system.

Although the tray hedge system did not constitute part of the experiment framework, a simple comparison with field hedges was evaluated to determine whether differences in rooting occurred (Figure 4.1) when the hedges were fertigated with the same nutrient solution (Mondi Orange fertiliser). A simple analysis of variance revealed that there were no significant differences between hedge type (tray vs. field hedge), but differences did exist between clones in terms of variation in rooting at the 5% level.

Figure 4.1 Comparison of field and tray hedge systems in terms of rooting performance (cutting date: 19/10/2000; assessment date: 19/12/2000)



In the experiment which follows, the objective was to compare the effect of three hydroponic nutrient solutions on the growth of *E. grandis x E. nitens* clonal hedge plants, their effect on rooting performance, and hedge plant sustainability. Hydroponica® was selected as the control solution for comparison with Mondi Orange and Mondi MM4 nutrient feeds. The latter two fertilisers are currently used in Mondi nurseries for the fertilising of seedlings (Orange) and for the fertigation of clonal field hedges (MM4). Both formulations have been tested extensively in nursery trials.

A further objective was to test the effects of artificial lighting on the growth of hedge plants in recirculating hydroponic units and whether there was any rooting response to reduced light intensity and reduced spectral distribution.

4.2 MATERIALS AND METHODS

4.2.1 Systems design

The trial was planted on 22/6/2000 and all experimentation was carried out at the Mondi Forests Mountain Home Nursery, KwaZulu-Natal. Three separate 'modified' NFT recirculating units were designed and built. The units were of the same design and materials as the previous trial (Chapter 3) and were 3.0 m x 1.2 m in dimension, and manufactured from 32 mm square mild steel tubing (Figure 4.2). The 'trough' system comprised 4 x 110 mm open plastic gutter sections braced to the frame. The gutter lengths were painted with black enamel to darken the root growth zone and lined with capillary matting to ensure the even distribution of liquid feed. Liquid fertilisers were circulated using a submersible pump (maximum volume 1400 L/hr to a maximum head of 1.8 m) installed in a 180 L plastic tank. Unequal cyclic timers were fitted to the individual units. The cyclic timers were set to run for fifteen minutes with an off-time interval of two hours. Timers were linked to an electronic clock that disrupted power supply at 18h00 and reconnected at 06h00.

Artificial lighting was fitted to each hydroponic unit and consisted of a 36 W new generation Gro-lux® fluorescent tube, one 40 W Flow-lux® tube, one 150 W incandescent bulb and two 150 W mercury globes. On the advice of a master electrician and a Phillips lighting consultant, it was believed that this combination would adequately cover the required light spectrum from 400 nm to 700 nm. An additional reflective shield was installed to direct the light more efficiently.

Figure 4.2 Side view of experimental hydroponic unit showing different substrates

4.2.2 Test clones

The three Mondi clones used in the first experiment (Chapter 3) were included in the trial. Their initial selection was based on mean rooting performance. The root collar diameter (mm), shoot length (cm) and number of whole leaves were measured for all planted ramets. This data set was used to determine whether stock plants were uniform at planting in order to reduce bias at the first coppice collection.

4.2.3 Substrate

Three different media were tested: Umgeni sand, 6.2 mm gravel, and horticultural grade perlite, whilst one block consisted of the original greenhouse media mixture (comprising 70% vermiculite, 20% perlite and 10% coconut-coir) and was identified as the 'NFT type'. Sand and gravel were sterilised with dilute formaldehyde (30%), rinsed thoroughly and poured immediately into the gutters to reduce contamination (Figure 4.2). The gutters were washed with Jik® (sodium hypochlorite) at a dilution rate of 5 mL Jik® per 5 L water, and well rinsed with clean water.

4.2.4 Experimental design and layout

The trial was established as a modified split-plot design. Substrates were randomised as whole plots and clones as subplots. The block contained four randomised whole plots; gravel, sand, perlite and NFT (insert). The whole plots contained three clones (subplots) with eight ramets/clone randomised within each plot (plot = 24 ramets). The trial layout is illustrated in Appendix D.1.1.

4.2.5 Elemental composition of test solutions

The composition of the three fertilisers tested in the experiment are summarised in Table 4.1.

Table 4.1 Elemental composition of the three test nutrients

Element	SI. Unit	Mondi Orange	Hydroponica®	Mondi MM4
N	g/kg	120	57	21
P	g/kg	160	46	47
K	g/kg	101	280	228
Ca	g/kg	0	0	0
Mg	g/kg	6	0	30
Fe	mg/kg	690	690	690
B	mg/kg	440	440	440
Na	mg/kg	300		300
Zn	mg/kg	270	270	270
Mo	mg/kg	90	90	90
Cu	mg/kg	140	140	140
S	mg/kg	0	0	132
Mn	mg/kg	0	300	0

(NEILAND, 1997)

4.2.6 Cultural practices

Based on the results of the first experiment (Chapter 3), fungicide applications were introduced in an attempt to reduce the effect of powdery mildew (*Oidium eucalypti*) and prevent the reoccurrence of *Pythium*. Solution pH was maintained at a range of 6.2 to 6.6, whilst EC levels were maintained at 1.2-1.3 μS/cm. Solutions were replaced every seven days and reservoirs were well rinsed prior to being refilled. At each refill, 1 ml of Sporekill® (quartenary ammonium chloride) disinfectant was added to the solution to curb the proliferation of pathogens. A fungicide treatment was introduced using Calixin®, Sporgon® (a.i. Prochloraz manganese chloride) and Benlate® (a.i. Benomyl), sprayed on a rotational basis. Calixin® and Sporgon® (150 g/100 L water) were sprayed during alternate weeks to aid in the control of powdery mildew, whilst Benlate®, (a systemic fungicide) was sprayed weekly at 2 g/L as a general preventative spray.

4.2.7 Preliminary measurements

From 22/6/2000 to 12/7/2000, measurements of pH, EC, light intensity and solution temperature were recorded for the purposes of determining interactions between these variates. The role of light intensity is analysed under results and discussion.

4.2.8 Plant tissue analysis

All nutrient analyses were carried out by the Cedara Plant Laboratory. The technique used is summarised in the previous experiment (Chapter 3).

4.2.9 Stem cuttings

Stem cuttings were prepared from juvenile shoots from hydroponic mother plants as described in the previous experiment (Chapter 3).

4.3 RESULTS AND DISCUSSION

4.3.1 Effect of ramet dimensions on planting stock

Measurements of stock plants at establishment yielded the summary in Table 4.2

Table 4.2 Mean ramet dimensions at establishment

Fertiliser	Substrate	Shoot length cm	Shoot diameter mm	No. of leaves
Hydroponica®	Gravel	12.16	1.99	8.25
Hydroponica®	NFT	11.24	1.93	7.88
Hydroponica®	Perlite	11.65	1.82	8.59
Hydroponica®	Sand	12.73	1.99	8.38
Hydroponica®	**Mean**	**11.94**	**1.93**	**8.27**
Mondi MM4	Gravel	11.50	1.88	7.75
Mondi MM4	NFT	12.61	1.95	7.75
Mondi MM4	Perlite	11.76	1.84	7.88
Mondi MM4	Sand	11.98	1.93	7.71
Mondi MM4	**Mean**	**11.96**	**1.90**	**7.77**
Mondi Orange	Gravel	11.39	1.95	7.50
Mondi Orange	NFT	12.84	1.94	8.34
Mondi Orange	Perlite	12.44	1.95	8.13
Mondi Orange	Sand	11.77	1.97	8.84
Mondi Orange	**Mean**	**12.11**	**1.95**	**8.20**

Summary statistics (Table 4.3) revealed a fairly normal distributed test sample. The standard deviations and standard errors around the mean values were low whilst the low skewness values reaffirmed a normal distributed sample set.

SCAGEL *et al.* (1998) suggest that the size of nursery plants can be expressed in terms other than height and root collar diameter. The sturdiness ratio is another means of expressing the shoot to root relationship (Figure 4.3). It is the ratio of seedling height to the root collar diameter. The lower the value, the more sturdy the seedling and the less likely it will suffer damage from competing vegetation.

Figure 4.3 Illustration of a rooted cutting to describe the concept of a sturdiness ratio

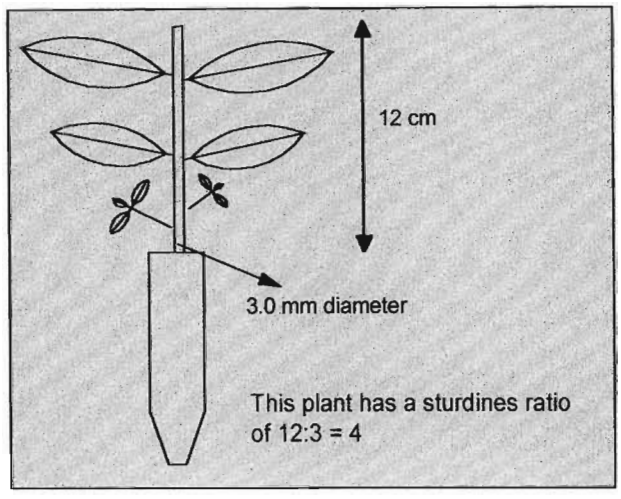


Table 4.3 Summary statistics for newly established ramets

	Diameter (mm)	Length (cm)	Sturdiness ratio (cm/mm)	No. leaves
Mean	1.93	12.01	6.23	8.08
Median	1.94	12.01	6.21	7.94
Minimum	1.69	9.05	5.03	6.75
Maximum	2.21	15.06	7.53	9.75
Range	0.52	6.01	2.50	3.00
Standard deviation	0.13	1.31	0.60	0.73
Standard error	0.02	0.22	0.10	0.12
Coefficient of variation	6.64	10.93	9.71	9.06
Skewness	-0.06	0.06	0.00	0.51

Analysis of variance (one-way, no blocking) showed significant differences between newly established clones for the number of leaves, stem length and sturdiness ratio (Table 4.4). However, diameter was not significantly different amongst clones. There was no difference between the variates at the substrate and fertiliser levels.

Table 4.4 Summary ANOVA data for newly planted hydroponic hedges

	No. leaves	Length (cm)	Diameter (mm)	Sturdiness ratio (cm/mm)
Clone ID_No.	** (F. pr. 0.005)	** (F. pr. <0.001)	n.s. (F. pr 0.127)	** (F. pr. 0.002)
Fertiliser type	n.s. (F. pr. 0.198)	n.s. (F. pr. 0.946)	n.s. (F. pr. 0.615)	n.s. (F. pr 0.840)
Substrate	n.s. (F. pr 0.542)	n.s. (F. pr. 0.829)	n.s. (F. pr. 0.456)	n.s. (F. pr. 0.611)

Significant at: * F = 0.05; ** F = 0.01; n.s. = not significant:; total d.f. = 35.

4.3.2 Effect of light on plant growth

Temperature, light and relative humidity (RH) are parameters that can be controlled in a hydroponic hedge system (DE ASSIS, 2001). For the period 24/8/2000 to 19/10/2000, the temperature and relative humidity immediately above the hydroponic frames were recorded every hour by a Hobo data logger. The maximum temperature recorded was 24.89 ^{0}C, the minimum temperature was 11.72 ^{0}C, whilst the mean was calculated at 17.88 ^{0}C. For the same data set, the maximum relative humidity recorded was 90.97%, the minimum RH was 46.89% and the calculated mean was 68.14%. A linear regression calculation showed a moderately weak inverse relationship (r = -0,642) between temperature and relative humidity.

A box plot was produced to give a visual comparison of the spread of data for the light intensity (Lux) measurements (Figure 4.4). Each box plot consists of a central box spanning the inter-quartile range of data for light intensity measurements (50% of the observations lie inside the box). The horizontal bar marks the median, and the 'whiskers' extend out to the largest and smallest observations. Data is summarised in Table 4.5.

Figure 4.4 Box plot of light intensity for experiment

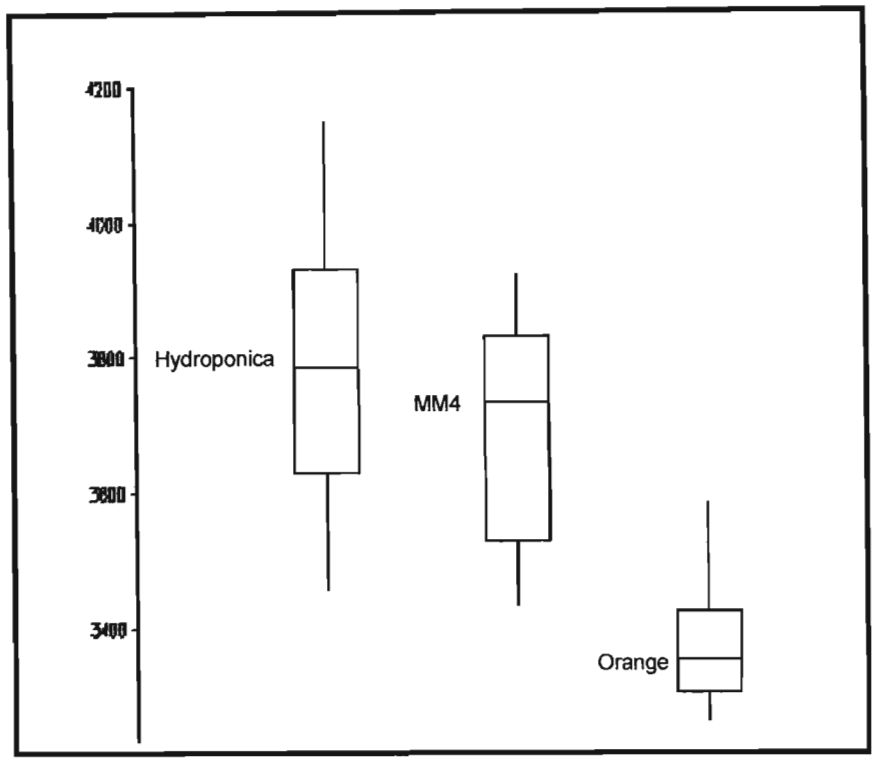


Table 4.5 Summary of light intensity statistics for newly established ramets

	Hydroponica®	Mondi Orange	Mondi MM4
Mean	3791.91	3381.00	3691.18
Median	3783.00	3353.00	3732.00
Minimum	3456.00	3262.00	3433.00
Maximum	4152.00	3586.00	3923.00
Range	696.00	324.00	490.00
Standard deviation	199.97	97.24	169.52
Standard error	60.29	29.32	51.11
Coefficient of variation	5.27	2.88	4.59
Skewness	0.07	0.84	-0.16

The statistical table, box plot and line graph (Figure 4.5) all indicated a wide range in light recordings and it was presumed that there were differences between the lighting intensity for the individual tables (especially for Mondi Orange fertiliser), even though they had exactly the same light fittings. To confirm these differences a series of t-tests (unpaired) were determined. The results are summarised in Table 4.6. The t-tests showed that there were significant differences in the distribution means of Mondi Orange (mean 3381 Lux) compared

to Hydroponica® (mean = 3792 Lux) and Mondi MM4 (mean = 3691 Lux), but there were no significant differences between Mondi MM4 and Hydroponica®.

Figure 4.5 Line plot of light intensity measured for the experiment

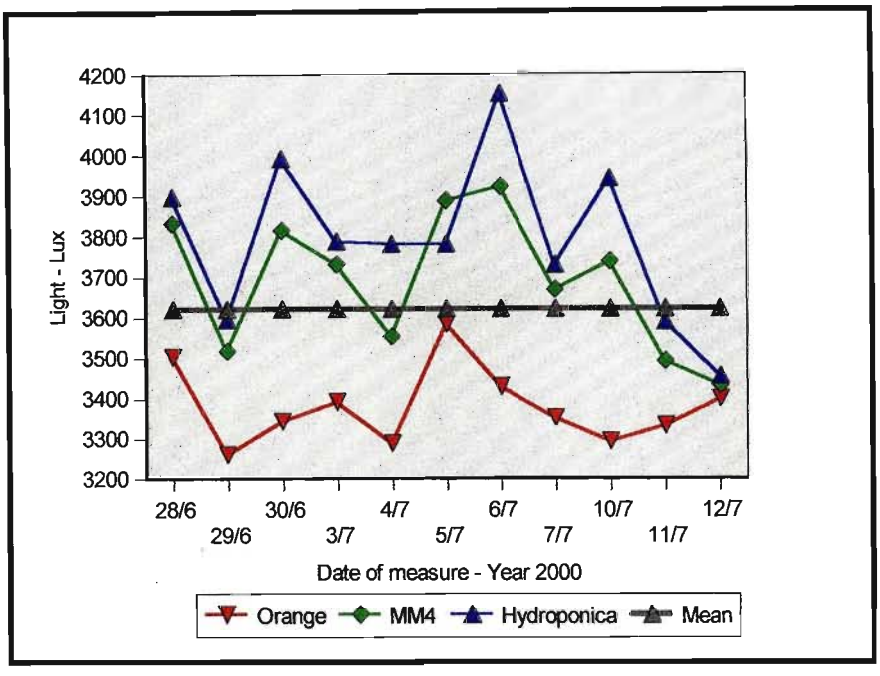


Table 4.6 Matrix of t-test values illustrating levels of significance for light intensity

	Hydroponica®	Mondi MM4	Mondi Orange
Hydroponica®	-	n.s. (p = 0.127)	** (p < 0.001)
Mondi MM4	n.s. (p = 0.127)	-	** (p < 0.001)
Mondi Orange	** (p < 0.001)	** (p < 0.001)	-

Significant at: * P = 0.05; ** P = 0.01; n.s. = not significant:; total d.f. = 20.

4.3.3 Plant tissue analysis

On 7/7/2000 the first set of *E. grandis x E. nitens* hybrid material was submitted to Cedara Plant Laboratory for dry mass analysis. The pooled data for fertiliser, clones and substrate are illustrated in Table 4.7 and Table 4.8.

Table 4.7 Plant tissue analysis across all treatments - 7/7/2000

Treat	N %	P %	K %	Ca %	Mg %	Na %	S%	C%	B mg/kg	Zn mg/kg	Cu mg/kg	Mn mg/kg
Hydroponica®-gravel	2.15	0.29	1.58	0.37	0.33	0.20	0.29	50.07	62.00	50.00	37.00	137.00
Hydroponica®-NFT	2.51	0.31	1.82	0.42	0.38	0.16	0.31	49.97	46.00	54.00	38.00	180.00
Hydroponica®-perlite	2.14	0.29	1.93	0.42	0.36	0.19	0.27	49.47	50.00	61.00	46.00	147.00
Hydroponica®-sand	2.41	0.30	1.89	0.41	0.41	0.18	0.30	49.91	45.00	55.00	37.00	140.00
Mean	**2.30**	**0.30**	**1.80**	**0.41**	**0.37**	**0.18**	**0.29**	**49.85**	**50.75**	**55.00**	**39.50**	**151.00**
Mondi MM4-gravel	2.16	0.29	1.87	0.41	0.39	0.19	0.29	49.39	53.00	45.00	35.00	134.00
Mondi MM4-NFT	2.18	0.30	1.98	0.41	0.41	0.18	0.30	49.12	54.00	49.00	25.00	150.00
Mondi MM4-perlite	1.93	0.26	1.68	0.39	0.35	0.19	0.24	48.92	50.00	48.00	29.00	110.00
Mondi MM4-sand	2.16	0.29	1.88	0.39	0.37	0.15	0.28	49.95	54.00	39.00	25.00	116.00
Mean	**2.11**	**0.28**	**1.85**	**0.40**	**0.38**	**0.18**	**0.28**	**49.34**	**52.75**	**45.25**	**28.50**	**127.50**
Mondi Orange-gravel	2.33	0.34	1.80	0.48	0.40	0.16	0.28	50.01	73.00	42.00	25.00	130.00
Mondi Orange-NFT	2.59	0.33	1.78	0.44	0.39	0.17	0.26	50.04	66.00	52.00	35.00	151.00
Mondi Orange-perlite	2.23	0.30	1.79	0.37	0.35	0.15	0.27	50.08	46.00	42.00	17.00	115.00
Mondi Orange-sand	2.57	0.32	1.77	0.42	0.38	0.16	0.29	50.91	52.00	44.00	23.00	140.00
Mean	**2.43**	**0.32**	**1.78**	**0.43**	**0.38**	**0.16**	**0.28**	**50.26**	**59.25**	**45.00**	**25.00**	**134.00**
Trial Mean	**2.28**	**0.30**	**1.81**	**0.41**	**0.38**	**0.17**	**0.28**	**49.82**	**54.25**	**48.42**	**31.00**	**137.50**

Table 4.8 Results of mean plant tissue concentrations across all hydroponic units

Summary statistic	C %	N %	P%	K%	Ca %	Mg %	S %	Na %	Mn mg/kg	Zn mg/kg	Mn mg/kg	Cu mg/k	B mg/kg
Mean	49.82	2.28	0.30	1.81	0.41	0.38	0.28	0.17	137.50	48.42	137.50	31.00	54.25
Median	49.96	2.21	0.30	1.81	0.41	0.38	0.28	0.17	138.50	48.50	138.50	32.00	52.50
Minimum	48.92	1.93	0.26	1.58	0.37	0.33	0.24	0.15	110.00	39.00	110.00	17.00	45.00
Maximum	50.91	2.59	0.34	1.98	0.48	0.41	0.31	0.20	180.00	61.00	180.00	46.00	73.00
Range	1.99	0.66	0.08	0.40	0.11	0.08	0.07	0.05	70.00	22.00	70.00	29.00	28.00
Standard deviation	0.53	0.20	0.02	0.11	0.03	0.03	0.02	0.02	19.17	6.40	19.17	8.26	8.60
Coefficient of variation (CV)	1.06	8.90	7.04	6.01	7.37	6.73	6.91	9.94	13.94	13.22	13.94	26.64	15.86
Skewness	0.10	0.19	0.07	-0.61	0.70	-0.31	-0.63	0.02	0.53	0.37	0.53	0.06	0.98

In the previous experiment, it was statistically proven that no significant differences existed between clones in terms of elemental concentration. The effect of genotype was therefore eliminated from the following statistical models and analysis data were pooled for substrate and fertiliser only.

To determine elemental differences between fertilisers and substrates, a series of ANOVA tests (one way, no-blocking) were run. A further ANOVA test (one way, randomised blocks) was run to establish whether there was any elemental variance for substrate plots within fertiliser blocks. This was not a fertiliser by substrate interaction, but a test of response to various stratum levels, i.e. block (fertiliser) and plot (substrate within fertiliser). The degrees of

freedom were low (d.f. =11) for an accurate assessment, but this was as a result of systems limitations on the number of replications possible. From plots of half-normal, residual values and a histogram of sample distribution, the sample set was found to be skewed towards the negative value. The ANOVA findings are summarised in Table 4.9.

Table 4.9 ANOVA of elemental differences between fertilisers, substrates, and substrate plots within fertiliser blocks

	C %	N %	P %	K %	Ca %	Mg %	S %	Na %	B mg/kg	Cu mg/kg	Zn mg/kg	Mn mg/kg
Fertiliser	**	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	*	n.s.
Substrate	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Fertiliser - substrate	**	**	*	n.s.	n.s.	n.s.	n.s.	**	n.s.	n.s.	n.s.	*

n.s. = not significant; * = significant at 5%; ** = significant at 1%

The ANOVA results showed no significant differences for nutrients between substrates. Only carbon was significant at the 1% level for differences between fertilisers, whilst phosphorus, copper and manganese showed significant variation at the 5% level. The ANOVA test for variability of substrate plots in fertiliser blocks revealed significant differences for carbon, nitrogen and phosphorus at the 1% level and copper, zinc, and manganese at the 5 % level.

4.3.4 Rooting results

After 73 days in the greenhouse, rooted cuttings were counted (Figure 4.6). Results are presented as actual rooting percentages and as derived numbers of rooted cuttings (Table 4.10 and Figure 4.7). It was deemed unnecessary to transform rooting data to arcsine values for radians.

For the experiment, the overall mean rooting was 51.99%. In terms of substrate, the rooting results were as follows :

- **Gravel NFT Perlite Sand**
- 53.78% 45.51% 54.25% 54.43%

In terms of fertilisers, the summary rooting results were as follows:

- **Hydroponica® Mondi MM4 Mondi Orange**
- 59.35 % 47.38% 49.25%

The mean rooting results by fertiliser and substrate were:

	Hydroponica®	Mondi MM4	Mondi Orange
• **Gravel**	59.42%	50.00%	51.92%
• **NFT**	59.60%	**35.50%****	41.43%
• **Perlite**	**60.83%** *	55.56%	46.36%
• **Sand**	57.53%	48.46%	57.30%

(* = Best substrate + fertiliser combination ** = Worst substrate + fertiliser combination.)

Figure 4.6 Mean ranked rooting performance by fertiliser type and substrate

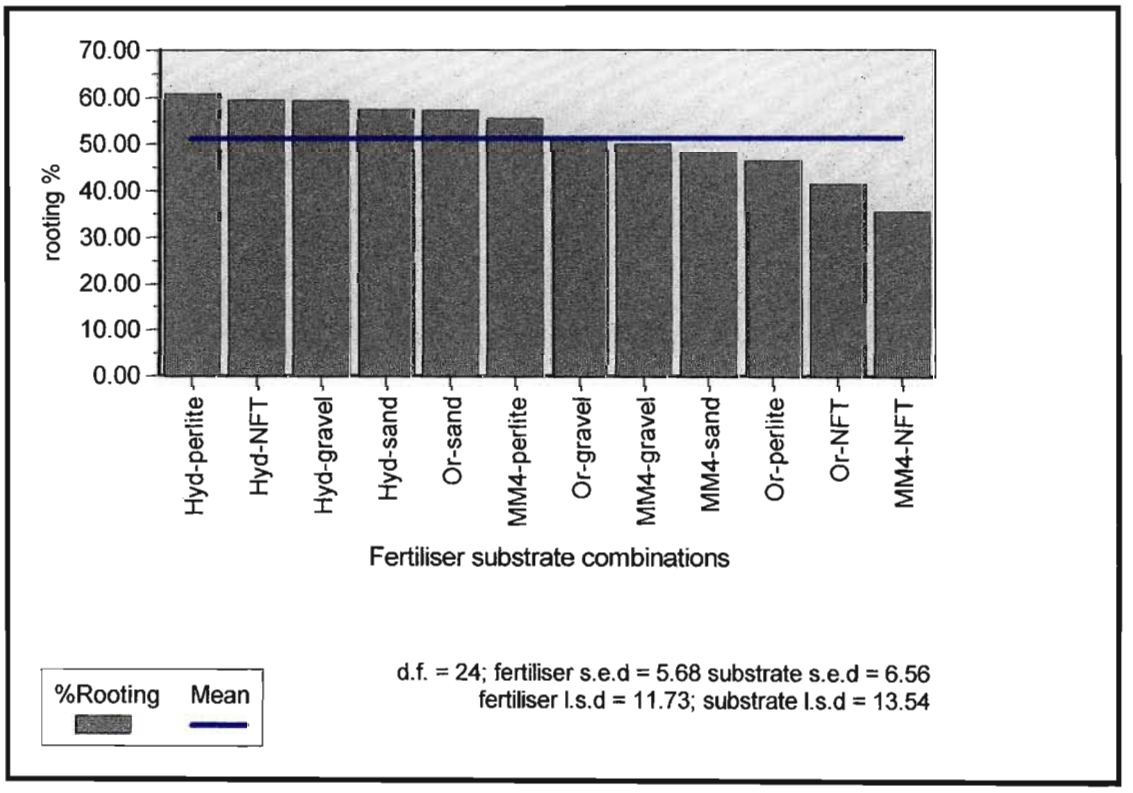
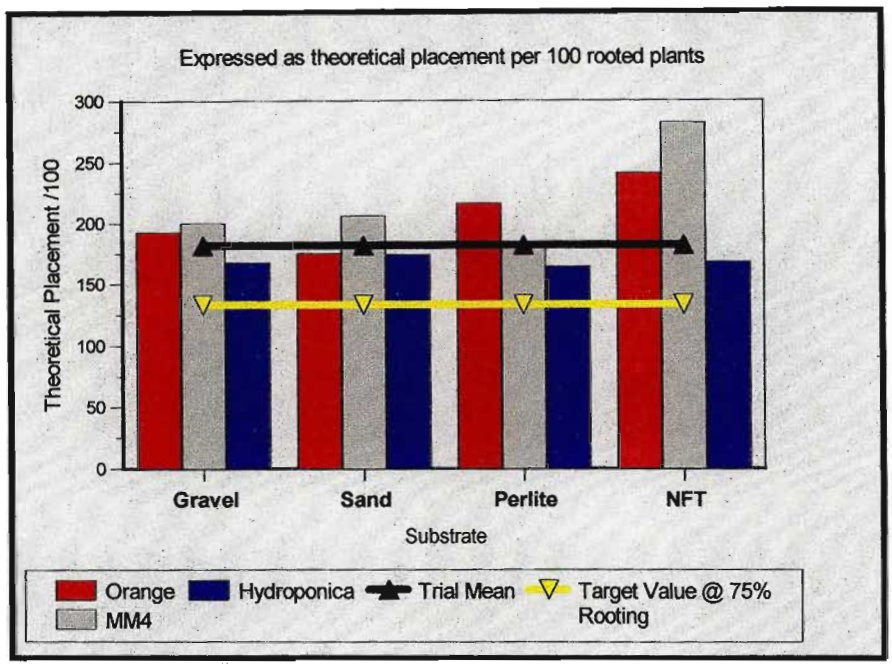


Table 4.10 Rooting data for 73-day-old cuttings expressed as actual rooting results and derived plant numbers (theoretical placement)

Fertiliser	Clone ID	Substrate	Cuttings placed	Cutting rooted	Root %	Harvest factor	Theoretical placement /100
Hydroponica®	GN156	gravel	52	26	50.00	0.500	200
Hydroponica®	NH000	gravel	59	43	72.88	0.729	137
Hydroponica®	GN107	gravel	27	13	48.15	0.481	208
Sum Value			**138**	**82**	**59.42**	**0.594**	**168**
Hydroponica®	GN156	NFT	51	26	50.98	0.510	196
Hydroponica®	GN107	NFT	49	29	59.18	0.592	169
Hydroponica®	NH000	NFT	51	35	68.63	0.686	146
Sum Value			**151**	**90**	**59.60**	**0.596**	**168**
Hydroponica®	NH000	perlite	49	27	55.10	0.551	181
Hydroponica®	GN107	perlite	28	20	71.43	0.714	140
Hydroponica®	GN156	perlite	43	26	60.47	0.605	165
Sum Value			**120**	**73**	**60.83**	**0.608**	**164**
Hydroponica®	NH000	sand	66	48	72.73	0.727	138
Hydroponica®	GN156	sand	73	38	52.05	0.521	192
Hydroponica®	GN107	sand	47	21	44.68	0.447	224
Sum Value			**186**	**107**	**57.53**	**0.575**	**174**
Mondi MM4	GN156	gravel	59	26	44.07	0.441	227
Mondi MM4	GN107	gravel	40	16	40.00	0.400	250
Mondi MM4	NH000	gravel	57	36	63.16	0.632	158
Sum Value			**156**	**78**	**50.00**	**0.500**	**200**
Mondi MM4	GN156	NFT	76	16	21.05	0.211	475
Mondi MM4	GN107	NFT	88	19	21.59	0.216	463
Mondi MM4	NH000	NFT	98	58	59.18	0.592	169
Sum Value			**262**	**93**	**35.50**	**0.355**	**282**
Mondi MM4	GN156	perlite	46	32	69.57	0.696	144
Mondi MM4	GN107	perlite	46	16	34.78	0.348	288
Mondi MM4	NH000	perlite	52	32	61.54	0.615	163
Sum Value			**144**	**80**	**55.56**	**0.556**	**180**
Mondi MM4	GN156	sand	40	24	60.00	0.600	167
Mondi MM4	GN107	sand	40	10	25.00	0.250	400
Mondi MM4	NH000	sand	50	29	58.00	0.580	172
Sum Value			**130**	**63**	**48.46**	**0.485**	**206**
Mondi Orange	NH000	gravel	32	17	53.13	0.531	188
Mondi Orange	GN156	gravel	41	25	60.98	0.610	164
Mondi Orange	GN107	gravel	31	12	38.71	0.387	258
Sum Value			**104**	**54**	**51.92**	**0.519**	**193**
Mondi Orange	GN107	NFT	33	9	27.27	0.273	367
Mondi Orange	GN156	NFT	48	21	43.75	0.438	229
Mondi Orange	NH000	NFT	59	28	47.46	0.475	211
Sum Value			**140**	**58**	**41.43**	**0.414**	**241**
Mondi Orange	NH000	perlite	35	17	48.57	0.486	206
Mondi Orange	GN156	perlite	41	22	53.66	0.537	186
Mondi Orange	GN107	perlite	34	12	35.29	0.353	283
Sum Value			**110**	**51**	**46.36**	**0.464**	**216**
Mondi Orange	GN156	sand	30	16	53.33	0.533	188
Mondi Orange	GN107	sand	33	17	51.52	0.515	194
Mondi Orange	NH000	sand	26	18	69.23	0.692	144
Sum Value			**89**	**51**	**57.30**	**0.573**	**175**

Figure 4.7 Mean derived rooting performance by fertiliser type and substrate



Analysis of variance (one-way, no blocking) was used to calculate rooting differences between clones, substrates and fertilisers. Interactions between fertiliser and substrate, clone and fertiliser, and clone and substrate were also calculated to determine significant rooting differences. The findings are summarised in Table 4.11.

Table 4.11 ANOVA summarising differences between fertilisers, substrates, clones and their interactions, in terms of rooting performance

	Clone	Fertiliser	Substrate	Fertiliser x Substrate	Clone x Fertiliser	Clone x Substrate
Root (F. pr.)	*	n.s.	n.s.	n.s.	n.s.	n.s.
s.e.d.	4.93	5.5	6.69	11.37	7.53	9.94

n.s. = not significant; * = significant at 5%; ** = significant at 1%

There were no significant rooting differences for any of the variates (Table 4.10), other than clonal differences at the 5% level.

4.3.5 Effect of nutrient concentration on rooting performance

The primary objective of the experiment was to identify those nutrients most strongly associated with rooting in hybrid eucalypts. Data from coppice material sent to Cedara Plant Laboratory for dry mass analysis is summarised in Table 4.12 as a comparison of mean values by treatment (fertiliser + substrate).

Table 4.12 Plant tissue analysis across all treatments. Cutting age = 73 days

Treat	N %	P %	K %	Ca %	Mg %	Na %	B mg/kg	Zn mg/kg	Cu mg/kg	Mn mg/kg	Protein %	Root %
Hydroponica®-gravel	2.92	0.66	1.84	0.51	0.23	0.16	163.00	58.00	32.00	176.00	18.25	59.42
Hydroponica®-NFT	2.96	0.70	2.21	0.39	0.25	0.14	107.00	63.00	32.00	192.00	18.51	59.60
Hydroponica®-perlite	2.99	0.70	1.93	0.47	0.24	0.15	137.00	66.00	34.00	230.00	18.71	60.83
Hydroponica®-sand	3.25	0.76	2.02	0.52	0.24	0.14	114.00	83.00	36.00	635.00	20.30	57.53
Mean	3.03	0.70	2.00	0.47	0.24	0.15	130.25	67.50	33.50	308.25	18.94	59.16
Mondi MM4-gravel	3.15	0.75	1.87	0.43	0.32	0.17	107.00	64.00	34.00	190.00	19.70	50.00
Mondi MM4-NFT	3.02	0.72	2.03	0.37	0.30	0.13	86.00	69.00	38.00	226.00	18.90	35.50
Mondi MM4-perlite	3.13	0.77	1.66	0.43	0.31	0.14	110.00	63.00	34.00	194.00	19.55	55.56
Mondi MM4-sand	3.01	0.75	1.70	0.42	0.30	0.14	138.00	65.00	35.00	319.00	18.79	48.46
Mean	3.08	0.75	1.81	0.41	0.31	0.14	110.25	65.25	35.25	232.25	19.23	45.38
Mondi Orange-gravel	3.11	1.03	1.51	0.56	0.31	0.23	147.00	67.00	34.00	208.00	19.41	51.92
Mondi Orange-NFT	3.29	1.01	1.58	0.52	0.30	0.19	133.00	66.00	39.00	298.00	21.00	41.43
Mondi Orange-perlite	3.35	1.09	1.51	0.51	0.27	0.21	147.00	54.00	31.00	188.00	20.95	46.36
Mondi Orange-sand	3.38	1.09	1.59	0.57	0.28	0.21	144.00	81.00	40.00	363.00	21.10	57.30
Mean	3.28	1.05	1.55	0.54	0.29	0.21	142.75	67.00	36.00	264.25	20.61	48.31
Trial mean	**3.13**	**0.84**	**1.79**	**0.47**	**0.28**	**0.17**	**127.75**	**66.58**	**34.92**	**268.25**	**19.60**	**50.95**

Using analysis of variance (one-way, no blocking), it was hoped to identify potential nutrient differences within fertilisers and substrates, prior to the calculation of a step-wise regression model (Table 4.13). The data set was limited by insufficient degrees of freedom (d.f.=11) and the standard error (s.e.d.) was noticeably larger because of the small size of the data set. Juvenile coppice material sent for dry mass analysis was grouped by substrate and fertiliser, with different clonal material being pooled within these blocks and plots. In retrospect, analysis by clone as a subplot would have been preferable and more valuable, but the prohibitive costs of nutrient analysis (R75.00 /sample) precluded this.

Table 4.13 ANOVA summary of foliar nutrient differences within fertilisers and substrate

	N %	P %	K %	Ca %	Mg %	Na %	B mg/kg	Cu mg/kg	Zn mg/kg	Mn mg/kg
Fertiliser	*	**	**	**	**	**	n.s.	n.s.	n.s.	n.s.
Substrate	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*
Fertiliser x Substrate	n.s.	n.s.	n.s.	**	n.s.	**	n.s.	n.s.	n.s.	n.s.

n.s. = not significant; * = 5% level of significance; ** = 1% level of significance

There were significant differences (5% level) between substrates for manganese effects, accounted for by sand (mean = 439 mg/kg versus overall mean 268 mg/kg). The last nutrient analysis data from July 2000 showed manganese not to be significantly different and manganese may have reached toxic levels due to the high cation exchange capacity (CEC) of sand, especially when fine silt is present.

There were surprising elemental differences amongst the fertilisers. Variation in phosphorus concentration was significant (1% level) for Mondi Orange in comparison to the last nutrient analysis run in July 2000, when it was only significantly different at the 5% level. This may be as a response to the warmer weather. Concentrations of sodium were significantly different (1% level), accounted for by Mondi Orange (mean = 0.21% versus overall mean = 0.17%). There was significant variation in nitrogen concentration at the 5% level and this was again due to Mondi Orange (mean = 3.28 % versus overall mean = 3.13%). Magnesium differences were highly significant (<1% level) due to Hydroponica® (mean = 0.24% versus overall mean = 0.28%), and potassium showed significant concentration variation (1% level) due to Mondi Orange (mean = 1.55% versus overall mean = 1.79%). Calcium concentrations were significantly different (1% level), explained once again by Mondi Orange (mean = 0.54 % versus overall mean = 0.48%).

A more complex ANOVA to determine the effect of levels of strata on differences was run. Variation between substrate plots, within fertiliser blocks, (not a substrate by fertiliser interaction) revealed significant differences (1%) in concentration for calcium (accounted for by NFT) and for sodium (accounted for by gravel).

Two separate linear regression outputs were calculated, to identify the nutrients that impacted on rooting performance. The first regression output is summarised in Table 4.14.

Table 4.14 Linear regression results for interaction of nutrients with rooting %

	N %	P %	K %	Ca %	Mg %	Na %	B mg/kg	Cu mg/kg	Zn mg/kg	Mn mg/kg
Correlation (*r*)	-0.21	-0.26	-0.23	0.26	-0.61	-0.07	0.34	-0.37	0.15	0.13
F pr.	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.
Std error	8.16.	8.05	8.12	8.05	6.61	8.31	7.85	5.3	8.24	8.26

n.s. = not significant; * = significant at 5% level; ** = significant at 1% level

A multiple linear regression model was determined for the relationship between rooting and the various nutrients to identify those with the greatest interaction. Utilising all the elements as explanatory variables, the model could account for 57.8% of the variance.

To produce a more explanatory model of the relationship of rooting and elements it was necessary to reduce the number of variables. For the data set, the model resulting from step-wise regression contained just two explanatory variables, **copper** and **zinc**. The correlation between copper and zinc was moderate ($r = 0.763$) and need not preclude the two

variables from appearing together. The resulting equation for the model was: **rooting % = 93.9 - 2.797 Cu + 0.836 Mg**. The percentage variance decreased from 57.8% for the 10 variable model to 39.0% for the two-variable model. Overall the model was a poor fit.

4.3.6 Fluctuation in nutrient concentration

A comparison of nutrient data, from clone banks and rooted cuttings, measured over a 103 day interval (results from July 2000 and October 2000), revealed changes in the concentration of elements over the period (Figure 4.8; Figure 4.9 and Table 4.15).

Figure 4.8 Macro-element concentration change over a 103 day period

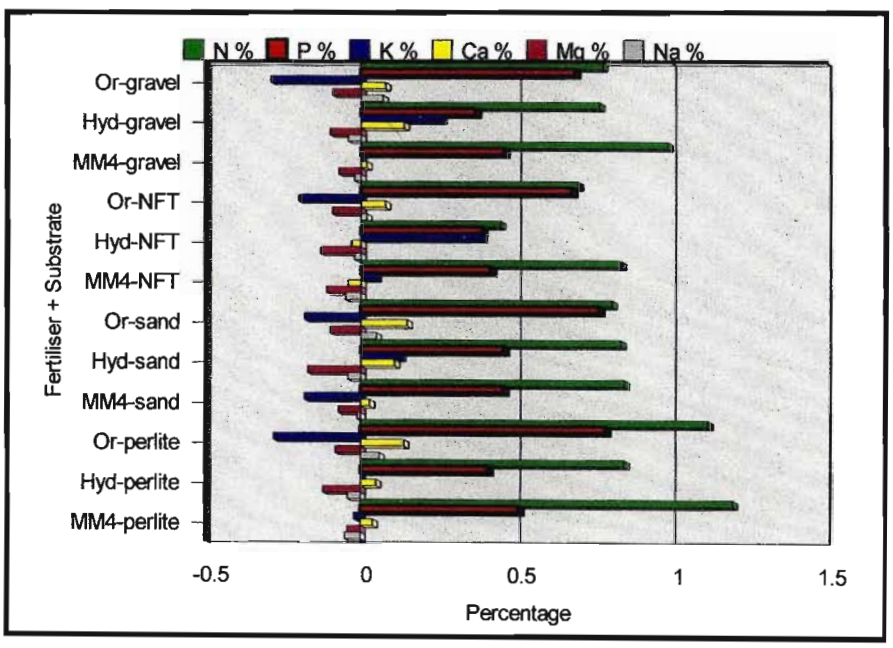


Figure 4. 9 Micro-element concentration change over 103 day period

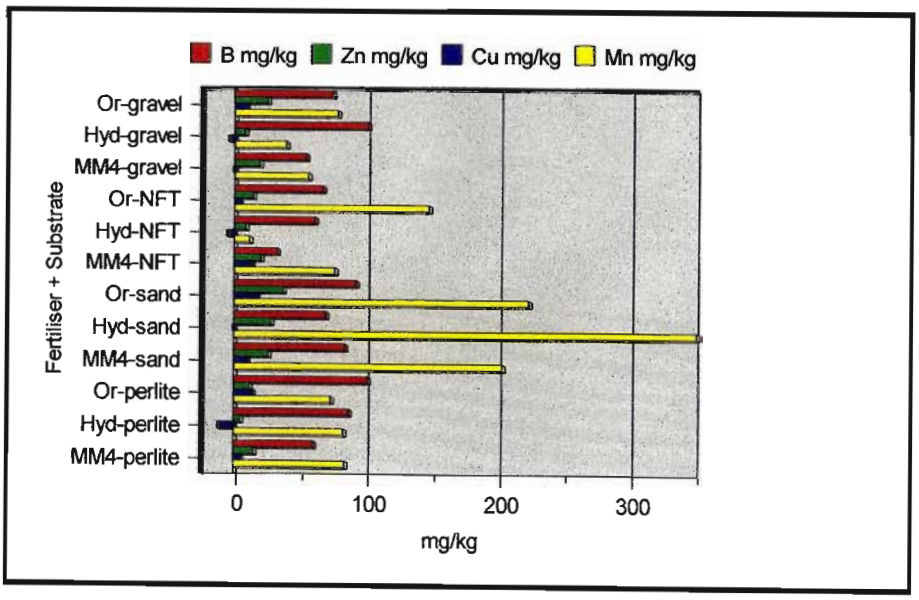


Table 4.15 Element concentration changes over 103 days

Fertiliser + substrate	Month	N %	P %	K %	Ca %	Mg %	Na %	B mg/kg	Zn mg/kg	Cu mg/kg	Mn mg/kg
Mondi Orange-gravel	Oct	3.11	1.03	1.51	0.56	0.31	0.23	147	67	34	208
	Jul	2.33	0.34	1.8	0.48	0.4	0.16	73	42	25	130
Difference		**0.78**	**0.69**	**-0.29**	**0.08**	**-0.09**	**0.07**	**74**	**25**	**9**	**78**
Hydroponica®-gravel	Oct	2.92	0.66	1.84	0.51	0.23	0.16	163	58	32	176
	Jul	2.15	0.29	1.58	0.37	0.33	0.2	62	50	37	137
Difference		**0.77**	**0.37**	**0.26**	**0.14**	**-0.1**	**-0.04**	**101**	**8**	**-5**	**39**
Mondi MM4-gravel	Oct	3.15	0.75	1.87	0.43	0.32	0.17	107	64	34	190
	Jul	2.16	0.29	1.87	0.41	0.39	0.19	53	45	35	134
Difference		**0.99**	**0.46**	**0**	**0.02**	**-0.07**	**-0.02**	**54**	**19**	**-1**	**56**
Mondi Orange-NFT	Oct	3.29	1.01	1.58	0.52	0.3	0.19	133	66	39	298
	Jul	2.59	0.33	1.78	0.44	0.39	0.17	66	52	35	151
Difference		**0.7**	**0.68**	**-0.2**	**0.08**	**-0.09**	**0.02**	**67**	**14**	**4**	**147**
Hydroponica®-NFT	Oct	2.96	0.7	2.21	0.39	0.25	0.14	107	63	32	192
	Jul	2.51	0.31	1.82	0.42	0.38	0.16	46	54	38	180
Difference		**0.45**	**0.39**	**0.39**	**-0.03**	**-0.13**	**-0.02**	**61**	**9**	**-6**	**12**
Mondi MM4-NFT	Oct	3.02	0.72	2.03	0.37	0.3	0.13	86	69	38	226
	Jul	2.18	0.3	1.98	0.41	0.41	0.18	54	49	25	150
Difference		**0.84**	**0.42**	**0.05**	**-0.04**	**-0.11**	**-0.05**	**32**	**20**	**13**	**76**
Mondi Orange-sand	Oct	3.38	1.09	1.59	0.57	0.28	0.21	144	81	40	363
	Jul	2.57	0.32	1.77	0.42	0.38	0.16	52	44	23	140
Difference		**0.81**	**0.77**	**-0.18**	**0.15**	**-0.1**	**0.05**	**92**	**37**	**17**	**223**
Hydroponica®-sand	Oct	3.25	0.76	2.02	0.52	0.24	0.14	114	83	36	635
	Jul	2.41	0.3	1.89	0.41	0.41	0.18	45	55	37	140
Difference		**0.84**	**0.46**	**0.13**	**0.11**	**-0.17**	**-0.04**	**69**	**28**	**-1**	**495**
Mondi MM4-sand	Oct	3.01	0.75	1.7	0.42	0.3	0.14	138	65	35	319
	Jul	2.16	0.29	1.88	0.39	0.37	0.15	54	39	25	116
Difference		**0.85**	**0.46**	**-0.18**	**0.03**	**-0.07**	**-0.01**	**84**	**26**	**10**	**203**
Mondi Orange-perlite	Oct	3.35	1.09	1.51	0.51	0.27	0.21	147	54	31	188
	Jul	2.23	0.3	1.79	0.37	0.35	0.15	46	42	17	115
Difference		**1.12**	**0.79**	**-0.28**	**0.14**	**-0.08**	**0.06**	**101**	**12**	**14**	**73**
Hydroponica®-perlite	Oct	2.99	0.7	1.93	0.47	0.24	0.15	137	66	34	230
	Jul	2.14	0.29	1.93	0.42	0.36	0.19	50	61	46	147
Difference		**0.85**	**0.41**	**0**	**0.05**	**-0.12**	**-0.04**	**87**	**5**	**-12**	**83**
Mondi MM4-perlite	Oct	3.13	0.77	1.66	0.43	0.31	0.14	110	63	34	194
	Jul	1.93	0.26	1.68	0.39	0.35	0.19	50	48	29	110
Difference		**1.2**	**0.51**	**-0.02**	**0.04**	**-0.04**	**-0.05**	**60**	**15**	**5**	**84**

NB: Nutrient concentration change reflected as difference between clone bank and rooted plants (October = clone bank nutrient status; July = rooted cutting nutrient status).

To understand the changes within element concentrations over the period of measurement (103 days), a simple ANOVA test (one-way, no blocking) was run (Table 4.16).

Table 4.16 ANOVA for change in nutrient concentration of rooted cuttings over 103 days

	N %	P %	K %	Ca %	Mg %	Na %	B mg/kg	Cu mg/kg	Zn mg/kg	Mn mg/kg
F. pr.	**	**	n.s.	**	**	n.s.	**	n.s.	**	**
Std error	0.07	0.05	0.07	0.02	0.01	0.01	6.96	2.52	3.02	38.00
Change in concentration	+	+	-	+	-	-	+	+	+	+

n.s. = not significant; * = significant at 5% level; ** = significant at 1%

There were significant changes (1% level) in elemental concentrations for seven out of the ten elements, and only potassium, copper and sodium were not significant (Table 4.16). The change in direction of concentration (increasing or decreasing) is also shown (Table 4.16). The assumption of the above test results is that there were significant changes in nutrient concentration of cuttings over the 103 day period and this may have affected rooting.

To explain more of the variation in differences in nutrient concentration, a more complex two-way ANOVA (in randomised blocks) was run. With data sets as block strata and fertiliser and substrates as plots (main effects), it was possible to calculate and identify the variation of the plots within the blocks and the resultant interactions over the 103 day period (Table 4.17).

Table 4.17 Two-way ANOVA test for change in nutrient concentration with rooting

	N %	P %	K %	Ca %	Mg %	Na %	B mg/kg	Cu mg/kg	Zn mg/kg	Mn mg/kg
Fertiliser	**	**	*	*	*	n.s	n.s.	n.s	n.s	n.s
Substrate	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
Fertiliser x Substrate	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s

n.s. = not significant; * = significant at 5%; ** = significant at 1%

The more inclusive ANOVA test showed that the original simple one-way test did not sufficiently account for the variation between the data sets over the period of time, and could have lead to a wrong conclusion. The consideration of block, plots and the interactions between plots, changed the output somewhat. The only significant differences in element concentration over the 103 day period were to be identified amongst the macro-elements, nitrogen, phosphorus, potassium, calcium and magnesium, and only at the fertiliser level. Differences at the substrate, and fertiliser by substrate levels were not significant. Micro-element concentrations did not significantly vary within the described model at any of the different defined levels.

4.4 CONCLUSIONS

Hedge plant dimensions showed significant differences between clones in terms of number of leaves, stem length and sturdiness ratio. Diameter was not significantly different amongst clones and there were no ramet size differences at the substrate and fertiliser level. WILSON (1993) suggests that the size of the stem of the cutting may contribute to within-shoot variation in rooting ability as basal cuttings are likely to be thick and internode length, which is easily reflected in cutting length, may also vary along the shoot.

The effect of ramet dimensions at establishment on rooting was not compared, but experience from the planting of field hedges has shown that plants with root collar diameters less than 3-4 mm are more prone to mortality as a result of adverse climatic conditions and thus affect hedge stocking and create variation in plant sizes.

Statistical comparison showed that light measured over the hydroponic test unit for Mondi Orange fertiliser recorded lower intensities (Lux) than that received by the other two units (Table 4.5). This anomalous situation could not be clearly explained as all units were fitted with the exact combination of supplementary lighting. For this study to have accounted more fully for the effect of light on hydroponically sustained eucalypt hedge plants, photosynthetic active radiation (PAR) should have been measured. However, the measurements do indicate that light intensity does affect the growth of coppice shoots (juvenile growth) to some extent and may adversely affect rooting.

VIEITEZ *et al.* (1998) note that light intensity can strongly influence cutting productivity and rooting by reducing or increasing endogenous phenolic substances that act as rooting inhibitors or promoters, depending upon the concentration in the plant tissue and the species involved. HANSEN (1987) believes that photoperiod combined with temperature can influence the rooting predisposition of shoots. Lighting can also influence rooting by controlling internal levels of carbohydrates. Etiolation of mother plants can affect rooting and root number which, depending on species, may increase or decrease. NANDA *et al.* (1971) suggest that this variation could be linked to carbohydrate production and HANSEN (1987) believes it is linked to the accumulation and transport of auxins. KUBOTA *et al.* (2001) suggest that one of the advantages of vegetative propagation under artificial light conditions is that rates of normal plant growth and multiplication can be precisely controlled, and therefore rates in each propagation cycle can be predicted by using a model.

The importance of lighting in South African hydroponic production systems needs to receive greater attention. Whereas in the Northern Hemisphere, the problem of insufficient light during winter can be improved through artificial lighting, it is more probable that under South African conditions, we are receiving too high a level of daily insolation that negatively affects rooting, but not necessarily vegetative growth. An etiolated hedge plant may accumulate higher concentrations of carbohydrates in the stem, which in turn could benefit rooting. This assumption needs to be tested by screening hydroponic beds with varying shade cloth densities. It may prove more practical to screen the hydroponic hedge plants with a dense shadecloth (75%) a week prior to the harvesting of coppice to induce an etiolated effect.

NEWTON *et al.* (1996), through their recent research into the vegetative propagation of tropical hardwoods, have highlighted a number of different pre-severance sources of variation in rooting ability of leafy stem cuttings. In particular, rooting is influenced by the position of the cutting within the shoot, the position of the shoot within the stock plant canopy and the number of shoots on the stock plant. Many of these positional effects may be attributable to the pre-severance light experienced by the cutting. The lower rooting percentages often associated with higher irradiance have been attributed to excessive carbohydrate accumulation. However, the light intensity during growth also influences aspects of cutting morphology, such as stem length and specific leaf area.

WILSON (1998) notes that in *E. globulus* in winter, supplemental lighting (SL) to extend the day-length in the glass house markedly increased survival. In the absence of SL, shade throughout the day on clear days was prejudicial to the rooting of *E. globulus* in terms of survival and rooting. The optimum appeared to be light shade during the mid-part of clear days.

The spectral quality of light is known to have a major effect on plant growth, development and morphology. In particular, a reduction in the ratio of red (660 nm) to far-red (730 nm) wavelengths (R:FR) has been shown to increase the stem elongation rate, specific leaf area (SLA) and relative growth rate. It also strengthens apical dominance by reducing lateral branching and influences rates of gaseous exchange (NEWTON *et al.*, 1996). HOAD *et al.* (1992) found that in *E. grandis*, higher rooting percentages recorded under lower R:FR ratios were associated with increased cutting lengths, higher specific leaf areas and higher rates of photosynthesis per unit leaf area during propagation. This relationship may be attributable to the capacity of the stem to store carbohydrates produced both pre- and post-severance.

NEWTON *et al.* (1996) report that lower R:FR ratio treatments resulted in higher rates of stem elongation in *Triplochiton scleroxylon* and *Terminalia spinosa*. Similar responses have been recorded with a number of tree species, including *Pinus radiata*, *Agathis australis*, *E. grandis* and *Acacia tortilis*. The stem elongation rate for *T. scleroxylon* under a R:FR ratio of 1.6 was found to be more than twice that under a ratio of 6.3 as a result of internode elongation. MORGAN *et al.* (1978) suggest that such responses can be interpreted as a shade avoidance mechanism and may be attributed to phytochrome-mediated regulation of gibberellin transport across cell membranes.

NEWTON *et al.* (1996) suggest that a number of tentative inferences can be made for the management of stock plants in the production of cuttings. Stock plant growth environments with lower R:FR ratios may be preferable, as higher rooting percentages are likely to result, particularly if cuttings with a fixed number of nodes are taken. Apart from advantages in terms of carbohydrate storage, longer cuttings also have the advantage of keeping the leaf free of the rooting medium. The appropriate pre-severance growth environment will also depend on the propagation environment in which the cuttings are placed.

LOU (1987) hydroponically cultured *Morus* sp. cuttings in the shade, in direct light, or in the dark followed by light (D/L) and given 50% or twice the normal nitrogen treatment. The growth of cuttings kept in the light was more rapid than that of cuttings in the other groups, regardless of the nitrogen treatment. The rate of photosynthesis of shaded cuttings was significantly lower than that of the other cuttings, whilst the photosynthetic rate of cuttings that were given the highest nitrogen treatment was slightly higher than those given the lowest. Water uptake per plant was about 10-20 ml per day in each group and increased with the growth of new shoots. There was a tendency in (D/L) cuttings to absorb more water than those kept in the light; shaded cuttings tended to absorb the least. The absorption of nitrogen, phosphorus and potassium by plants in the dark decreased, but it increased rapidly and even exceeded that of cuttings in the light, after transfer into natural light conditions. Plants in the highest nitrogen group absorbed twice the amount of nitrogen absorbed by the lowest nitrogen group, but less phosphorus and potassium.

In the experiments of this study, ANOVA tests showed no differences in the nutrient status of cuttings at substrate level, whilst carbon, copper and manganese concentrations varied significantly amongst fertilisers. There were no differences in rooting response due to substrates which infers that cost of media would be a more decisive reason influencing choice. Fertilisers showed no significant differences in their effect on rooting and it would appear that as long as all of the essential elements are available in the correct ratios, and are of a pure and highly soluble form, most commercially available hydroponic fertilisers will suffice. The mean rooting response to fertiliser and substrate combinations revealed that Hydroponica® combined with dolomitic gravel produced the best rooting (60.83%) in the modified NFT unit whilst a combination of Mondi MM4 fertiliser and NFT inserts produced the poorest response (35.50%).

There were a surprising number of significant differences within the fertiliser group for different elements. Nitrogen, phosphorus, potassium, sodium and calcium showed significant

variation and this could be accounted for by Mondi Orange, whilst magnesium proved to be highly significant (<1%) as a result of Hydroponica® fertiliser. It is worthwhile noting that Mondi Orange was initially formulated for the raising and hardening of forestry seedlings, whilst Mondi MM4 was specifically prepared as soluble nutrient feed for field hedges growing on the sandy dry Fernwood soils of Zululand. The only 'true' hydroponically formulated feed was Hydroponica® which proved to be the best test nutrient.

The identification in this experiment of copper and zinc interacting with rooting differs from the previous findings evaluated in January 2000, where calcium and magnesium had the strongest correlation with rooting. No clear or logical reason can explain why there should be such a large shift, however, it is interesting that previously, two macro-elements responded to rooting whilst now two micro-elements showed the strongest linear interaction. This could be linked to a physiological change in nutrient requirements as a result of increasing ramet maturity.

Changes within element concentrations over a 103 day interval revealed that fertiliser type affected the macro-elements nitrogen, phosphorus, potassium, calcium and magnesium. However, this change could not be directly linked to rooting differences and may have very little value. SVENSON *et al.* (1995) found that in *Euphorbia pulcherrima*, changes in selected macro- and micro-elemental concentrations coincided with early root initiation. Iron, copper and molybdenum concentrations increased in the basal portions of the unrooted stem cuttings during the root initiation stage, suggesting that concentrations of these elements may be important for early root primordia formation or other concurrent growth processes. Subsequent root primordia elongation was influenced by these elements and increased the concentrations of magnesium, manganese, boron and zinc.

In this experiment on eucalypt clones, manganese, boron and zinc increased in concentration and confirmed the findings of SVENSON *et al.* (1995). However, magnesium concentration decreased in the hybrid eucalypts as compared with what SVENSON *et al.* (1995) found in poinsettia. The multiple linear regression also identified zinc as affecting the rooting of hybrid eucalypts.

Moderate copper concentrations have increased root initiation and root elongation during *in vitro* rooting of birch micro-cuttings (SVENSON *et al.*, 1995). Copper increased in concentration over 103 days in this experiment, but only at the fertiliser level. However, it was also strongly associated with rooting response in the regression model.

Zinc can promote the formation of the auxin precursor, tryptophan, and the formation of auxin from tryptophan. Conversely, manganese acts as an activator of the IAA-oxidase enzyme system, and boron may enhance IAA-oxidase activity. (SVENSON *et al.*, 1995). HARTMANN *et al.* (1990) suggest that higher endogenous auxin levels are required for early root initiation than for later root development. SVENSON *et al.* (1995) postulate that if root initiation is related to the relative activity of IAA and IAA-oxidase, then rooting may be correlated with the relative zinc, manganese, and boron concentrations at the site of root initiation. An analysis of IAA and IAA-oxidase activity concurrent with selected micro-element concentrations during root initiation, is needed to confirm or reject the hypothesis that relative concentrations of macro- and micro-elements at the site of root initiation can influence the discrete developmental stages of adventitious root formation.

CHAPTER 5

RESPONSE OF CLONAL EUCALYPT HEDGE PLANTS TO SEVEN NUTRIENT SOLUTIONS IN A RECIRCULATING HYDROPONIC SYSTEM

5.1 INTRODUCTION

According to HASSIG (1986), there is a need to understand the metabolism of rooting on the whole cutting basis, rather than in the rooting zone alone. DICK and DEWAR (1992) state that the rooting of vegetative leafy cuttings involves the complex interaction of many processes. For this reason, the influence of carbohydrate status, nutrition, water and hormonal factors on root formation is poorly understood at the metabolic level. The initiation and development of adventitious roots involves many interactive processes, but experiments on rooting usually consider only a few of the most easily assessed factors known to influence rooting. The primary internal controls on the initiation and development of adventitious roots include the carbon, water and nutrient budgets of the cutting, as well as hormonal factors. These internal factors are affected by environmental influences on the cutting such as light intensity, air and soil temperatures, vapour pressure deficit and nutrient supply, both when the cutting is part of the stock plant and after it has been detached and placed in a propagation bed. The search for a single limiting 'rooting' factor is unlikely to succeed without an integrated approach that encompasses the physiological and environmental controls.

The objective of this experiment was to compare seven different fertiliser formulations and their effects on coppice production and rooting success of *E. grandis x E. nitens* clones. Although we accept that nutrition cannot be dealt with as a separate study from the other processes that affect rooting, it should be possible to identify and formulate a fertiliser feed that better suits the nutritional requirements of the *E. grandis x E. nitens* cross and improves the rooting vigour.

5.2 MATERIALS AND METHODS

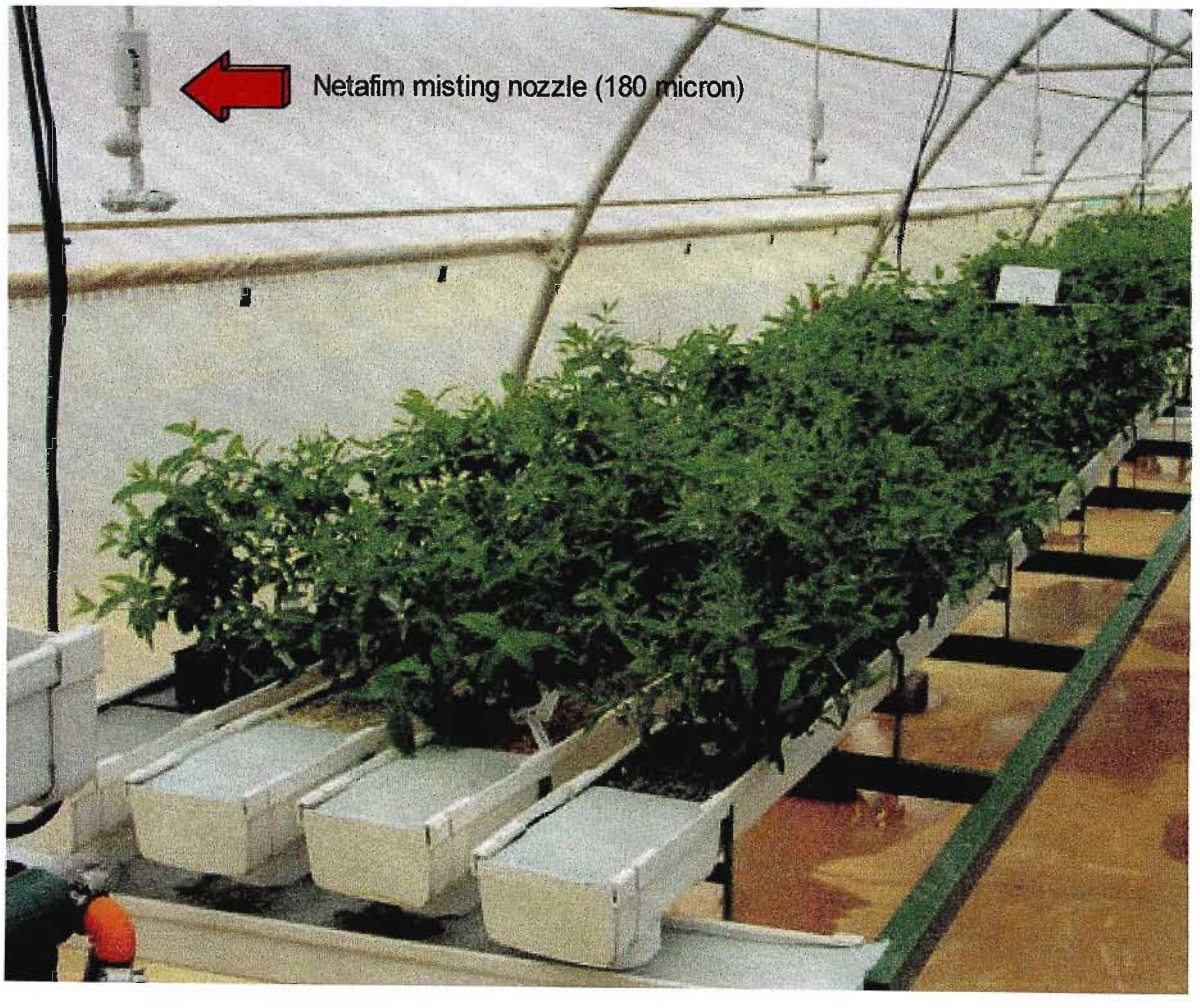
5.2.1 Systems design

The trial was planted on 18/6/2001 and all experimentation was carried out at the Mondi Forests Trahar Technology Centre, KwaZulu-Natal. Seven separate 'modified' NFT

recirculating units were designed and built (Figure 5.1). The units were of the same design configuration as those used in the two previous trials (Chapters 3 and 4).

No artificial lighting was necessary as all units were grown in a 8 x 30 m plastic covered tunnel with 40% shade cloth covering. Light readings in the tunnel exceeded 10 000 Lux on a clear day. Extractor fans were set to operate at 28 ^{0}C and no wet wall was present. Additional overhead misting lines were installed utilising the Netafim® misting nozzle (Figure 5.1) to aid in the cooling of the plants.

Figure 5.1 Side elevation of hydroponic units in tunnel at Trahar Technology Centre



5.2.2 Test clones

The three Mondi clones GN107, GN156 and NH000, were used for the trial.

5.2.3 Substrate

See Chapter 4.2.3 for description.

5.2.4 Experimental design and layout

The trial was established as a modified split-plot design. Substrates were randomised as whole plots and clones as subplots. The block contained four randomised whole plots; gravel, sand, perlite and NFT (insert). The whole plots contained three clones (subplots) with ten ramets/clone randomised within each plot (plot = 30 ramets). The combination of Hydroponica® nutrient feed and NFT was selected as the control because of its inclusion in all the trials to date.

5.2.5 Elemental composition of test solutions

Seven fertiliser solutions were tested in the experiment and included Hydroponica®, Murashige and Skoog (1962), Natgro®, Hoaglands ½ strength solution, Mondi Orange nutrient feed, Mondi MM4 feed, and Hydrofeed®. Freshly diluted nutrient solution samples were sent to the ARC in Nelspruit to determine the actual chemical content (Table 5.1). The solution strengths were maintained at 1100-1200 µS/cm and a pH of 5.8 to 6.7.

Table 5.1 Analysis of solutions at start of a single cycle - 06/02/2002

	pH	EC S/cm	TDS mg/l	Ca mg/l	Mg mg/l	K mg/l	Na mg/l	Cl mg/l	NO_3 mg/l	SO_4^{2} mg/l	Zn mg/l	Cu mg/l	Mn mg/l	Fe mg/l	B mg/l	NH_3 mg/l	P mg/l	N mg/l
Tap water	8.39	105	73.4	12.7	36.5	32	290	8.55	0-10	0	0.02	0.01	0	0.02	0.25	0	<0.05	<1
Hydroponica	6.08	1579	1105.3	89.0	45.0	240	390	13.70	100-250	<400	1.52	0.22	0.36	0.52	0.85	10-25	13.25	<1
M & S	6.37	2520	1764.0	58.3	12.2	263	380	76.00	250-500	0	2.21	0.02	1.39	0.37	0.45	100-200	8.15	53
Natgro	6.67	1382	967.4	68.1	38.0	192	375	29.40	100-250	<400	1.32	0.04	0.25	0.63	1.35	10-25	3.85	<1
Hoaglands	6.72	1416	991.2	166.0	25.7	125	355	9.69	100-250	0	0.52	0.01	0.36	0.36	0.25	0	7.60	<1
Orange	5.85	1345	941.5	64.2	11.6	116	365	10.50	100-250	0	1.17	0.10	0.29	0.4	0.7	100-200	140.00	4.21
MM4	6.62	2460	1722.0	67.9	50.3	421	390	23.10	50-100	<800	0.42	0.01	0.24	0.08	0.45	0-10	1.15	<1
Hydrofeed	6.53	1364	954.8	67.6	19.8	170	370	11.90	250-500	0	0.86	0.03	0.22	0.93	0.65	25-50	6.90	<1

NO_2^- = 0 mg/l; Blue = highest value; red = lowest value (highest and lowest values exclude tap water)

5.2.6 Cultural practices

The same protocols were applied as in the previous experiment (See Chapter 4.2.5).

5.2.7 Preliminary measurements

From 18/6/2001 to 24/6/2002, a Hobo data logger was used to record ambient temperature and relative humidity (RH) to determine whether the correlation between temperature and relative humidity continued to be inversely related as was recorded in the previous experiment (Chapter 4).

5.2.8 Plant tissue analysis

All nutrient analyses were carried out by the Cedara Plant Laboratory. The laboratory techniques are summarised in Chapter 3.

5.2.9 Stem cuttings and harvest intervals

Stem cuttings were prepared from the juvenile shoots of hydroponic stock plants (Figure 5.2). Standard cutting dimensions, as described in Chapter 3, were applied. Sand bed heating was adjusted to 32 °C and extractor fans were set to activate at 28 °C ambient temperature. Cuttings were misted at eight to twelve second intervals every 25 minutes. Three separate harvests were placed (Table 5.2) and each batch of cuttings was set for 60 days in the propagating tunnel and 15 days in an unheated plastic covered structure (Figure 5.3).

Table 5.2 Dates of coppice harvest and evaluation of rooted plants

Harvest	Date placed	Date evaluated
Harvest 1	14/9/2001	28/11/2001
Harvest 2	20/11/2001	4/02/2001
Harvest 3	14/1/2002	28/3/2002

Figure 5.2 Coppice material prior to harvest

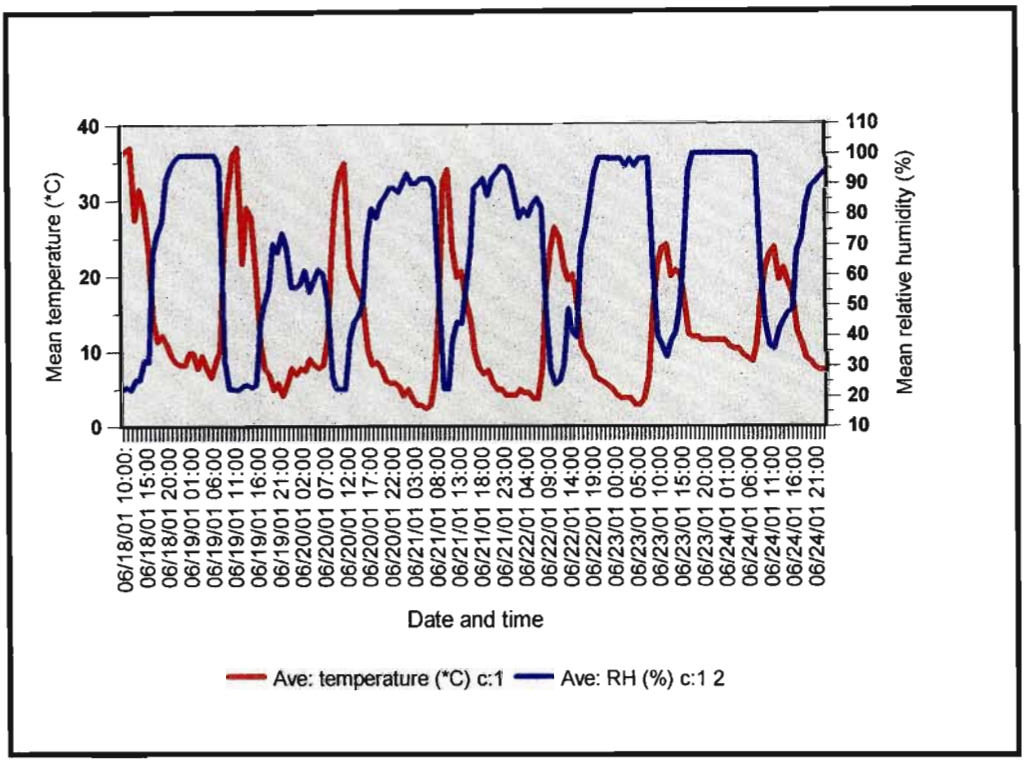
Figure 5.3 Cuttings on 9/11/2001 after 56 days in the greenhouse

5.3 RESULTS AND DISCUSSION

5.3.1 Relationship between temperature and relative humidity

For a period of six days (18/6/2001 to 24/6/2001), a data logger was placed in the plastic covered tunnel housing the hydroponic stock plants to measure temperature and relative humidity every hour. The recorded maximum temperature was 37 ^{0}C, the minimum temperature was 2.46 ^{0}C, whilst the mean was calculated at 13.29 ^{0}C. For the same data, the maximum relative humidity recorded was 99%, the minimum RH 22.5% and the calculated mean 69.45%. A linear regression calculation showed a strong inverse relationship (r = -0,861) between temperature and relative humidity. The model accounted for 73.9% of the variance. The linear equation for the relationship was: **RH% = 104.52(constant) - 2.639(x),** where x = temperature. It was of concern to observe the large temperature fluctuations which must have had a negative impact on the ramet's physiology and affected the ability to produce high quality coppice (Figure 5.4).

Figure 5.4 Inverse relationship between temperature and relative humidity in the hydroponic tunnel (date 18/6 - 24/6/2001)



5.3.2 Correlation of cutting dimensions and rooting performance

After 74 days (28/11/2001), the first harvested material was assessed. All rooted cuttings were measured to determine length of cutting (cm), and root collar diameter (RCD). It was postulated that cutting dimensions correlated strongly with rooting response. A multiple linear regression with rooting as the response variate and fitted terms, cutting length, sturdiness ratio (see Chapter 4) and root collar diameter, produced no strong correlation values. All correlation values (*r* values) were weak, ranging from 0.369 (RCD) to 0.398 (sturdiness ratio). It was concluded that rooting response cannot be determined from cutting length and root collar diameter, as the relationship was not significant. The best fitted model could only account for 14.8% of the variance. The mean rooting for the regression was 15.32%, the mean cutting length was 3.9 cm and the RCD was 1.46 mm.

5.3.3 Rooting performance as a response to fertiliser, clone and substrate at first harvest

From the first harvest, an ANOVA test (two-way, randomised blocking) showed that rooting differed significantly (1% level) at the clonal and fertiliser levels (5%), but that there was no significant differences at the substrate, clone by substrate, fertiliser, and clone by fertiliser

levels (Table 5.3). This confirmed that differences in rooting are strongly related to genotype and weakly responsive to different fertilisers.

Table 5.3 Summary ANOVA data for first harvest

	Clone ID_No.	Substrate	Clone x Substrate	Fertiliser	Clone x Fertiliser
Root %	**	n.s.	n.s.	*	n.s.

Significant at: * P=0.05; ** P= 0.01; n.s. = not significant:; total d.f. = 83.

The first harvest produced an overall rooting mean of 15.3%. This poor result is similar to that achieved from the first harvest in clonal field hedges, but is disappointing in comparison to the rooting results recorded in Chapters 3 and 4 where lighting was thought to be the limiting factor.

At the substrate level, rooting results were:

- **Gravel Sand Perlite NFT**
- 16.0% 15.3% 16.5% 13.5%

For fertilisers, the summary rooting results were:

- **Hydroponica® M&S Natgro ® Hoaglands Mondi Orange MM4 Hydrofeed ®**
- 11.2 % 15.4% 12.6% 25.3% 12.4% 10.6% 19.7%

The results of rooting in response to fertiliser and substrate showed the worst results to be from a combination of Hydroponica® and NFT, whilst the best results were from a Hoaglands solution and sand combination (Table 5. 4).

Table 5.4 Matrix of rooting percentages by fertiliser and substrate - first harvest, 28/11/2001

	Hydroponica®	M&S	Natgro®	Hoaglands	Mondi Orange	Mondi MM4	Hydrofeed®
Gravel	28.50	10.50	7.60	23.60	8.80	7.70	25.70
Sand	5.70	22.90	6.00	**30.30** **	17.90	8.90	15.10
Perlite	9.90	19.20	16.90	29.50	14.00	8.10	17.90
NFT	**1.00***	9.20	19.80	17.80	9.00	17.70	20.00

* = worst rooting result; ** = best rooting result.

5.3.4 Effect of nutrient concentration on rooting performance at first harvest

The effect of individual elements on rooting was a prime objective of this experiment. From analysis of variance (ANOVA one-way, no blocking), differences were sought for nutrients

within fertiliser and substrate groups, prior to the calculation of stepwise regression models. Juvenile coppice material sent for dry mass analysis was grouped by substrate and fertiliser, with different clones pooled within the blocks and plots. Tissue analysis comparisons using ANOVA tests, produced the output in Table 5.5.

Table 5.5 ANOVA of nutrient differences at the fertiliser and substrate level

	C %	N %	P %	K %	Ca %	Mg %	S %	Na %	B mg/kg	Cu mg/kg	Fe mg/kg	Zn mg/kg	Mn mg/kg
Fertiliser	**	**	**	**	**	**	**	**	n.s.	**	**	**	**
Substrate	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	**	n.s.	n.s.	n.s.	n.s.

n.s. = not significant; * = 5% ; ** = 1%; (d.f. = 27)

There were significant differences (1% level) for all elements between different fertilisers, except for boron which could not be accounted for. Murashige and Skoog (1962) solution accounted for significant differences in carbon, iron, manganese and nitrogen. Hoaglands solution (1/2 strength) accounted for the differences in calcium, phosphorus and zinc. Hydroponica® accounted for the elemental differences within copper and sodium, whilst Mondi Orange solution accounted for the significant elemental differences in potassium. Mondi MM4 was significantly different for the magnesium whilst Natgro® accounted for elemental concentration differences in sulphur.

There were no significant differences in elemental concentrations for any of the substrates, except for boron, and this was accounted for by perlite. A further ANOVA test (one way, no blocking) to determine differences in **rooting** as a response to fertiliser type, (Table 5.6) showed no significant differences (F. pr. 0.069).

A multiple linear regression model was run for the interaction between rooting and the various nutrients. The effect of substrate and fertiliser were excluded on the basis of analysis of ANOVA findings. Utilising all the elements as explanatory variables, the model could account for only 0.4 % (d.f. = 27) of the variance. Calcium was the only element to correlate (r = 0.505) moderately with rooting.

For the data set, the model resulting from step-wise regression contained just one explanatory mode, calcium. The equation for the interaction was: **rooting % = -3.29 + 40.0(Ca)**. The percentage model variance accounted for an increase from 0.4% for the 10 variable model to 22.6% for the single variable. Overall the model was a poor fit, but it is

noteworthy that calcium should be identified once again (Chapter 3) as interacting with rooting.

Table 5.6 Plant tissue analysis across all treatments (placed 14/09/2001, assessed 28/112001)

	Substrate	N %	P %	K %	Ca %	Mg %	Na %	S %	C %	B mg/kg	Zn mg/kg	Cu mg/kg	Mn mg/kg	Fe mg/kg	Root %
Hydroponica®	Sand	3.39	0.44	2.08	0.49	0.22	0.08	0.32	44.54	34	89	14	466	121	5.70
	Gravel	3.29	0.44	2.14	0.46	0.23	0.1	0.3	46.92	45	84	15	306	88	28.50
	Perlite	3.26	0.42	2.07	0.44	0.25	0.1	0.31	46.48	31	92	13	334	97	9.90
	NFT	3.08	0.4	2.12	0.4	0.25	0.11	0.3	47	48	84	13	348	89	1.00
	Mean	3.26	0.43	2.10	0.45	0.24	0.10	0.31	46.24	39.50	87.25	13.75	363.50	98.75	11.28
Murashige & Skoog	Sand	3.73	0.39	1.99	0.39	0.24	0.09	0.33	48.1	23	93	13	683	139	22.90
	Gravel	3.44	0.36	2.00	0.46	0.22	0.09	0.3	47.31	32	87	15	529	194	10.50
	Perlite	3.58	0.38	1.95	0.37	0.26	0.12	0.33	48.17	23	97	11	544	155	19.20
	NFT	3.26	0.36	2.00	0.39	0.24	0.14	0.3	48.45	33	102	10	507	215	9.20
	Mean	3.50	0.37	1.99	0.40	0.24	0.11	0.32	48.01	27.75	94.75	12.25	565.75	175.75	15.45
Natgro®	Sand	3.07	0.36	1.78	0.42	0.24	0.14	0.26	46.08	31	73	9	370	90	6.00
	Gravel	3.13	0.41	1.94	0.53	0.25	0.17	0.27	45.89	46	86	11	283	110	7.60
	Perlite	3.1	0.41	1.97	0.5	0.25	0.13	0.27	46.38	30	78	6	229	95	16.90
	NFT	2.94	0.36	2.07	0.4	0.25	0.14	0.26	45.65	44	73	9	265	127	19.80
	Mean	3.06	0.39	1.94	0.46	0.25	0.15	0.27	46.00	37.75	77.50	8.75	286.75	105.50	12.58
Hoaglands	Sand	3.04	0.38	1.67	0.73	0.23	0.1	0.3	46.7	32	64	11	213	179	30.30
	Gravel	3.08	0.35	1.61	0.65	0.24	0.13	0.25	47.75	32	55	8	191	107	23.60
	Perlite	3.27	0.39	1.78	0.66	0.27	0.12	0.28	47.02	27	74	8	219	82	29.50
	NFT	3.12	0.34	1.88	0.62	0.28	0.14	0.26	47.42	36	70	8	232	76	17.80
	Mean	3.13	0.37	1.74	0.67	0.26	0.12	0.27	47.22	31.75	65.75	8.75	213.75	111.00	25.30
Mondi Orange	Sand	3.43	0.42	1.55	0.48	0.23	0.16	0.31	47.77	36	112	11	554	103	17.90
	Gravel	3.02	0.49	1.56	0.51	0.24	0.15	0.26	46.96	45	84	11	302	116	8.80
	Perlite	3.08	0.47	1.53	0.42	0.26	0.18	0.25	45.35	35	97	9	323	120	14.00
	NFT	3.06	0.5	1.44	0.38	0.27	0.12	0.26	47.07	56	85	11	301	159	9.00
	Mean	3.15	0.47	1.52	0.45	0.25	0.15	0.27	46.79	43.00	94.50	10.50	370.00	124.50	12.43
Mondi MM4	Sand	3.27	0.42	1.61	0.34	0.32	0.09	0.3	47.52	34	80	8	302	78	8.90
	Gravel	3.21	0.42	1.76	0.44	0.31	0.11	0.3	47.07	34	87	11	281	97	7.70
	Perlite	3.23	0.4	1.65	0.36	0.34	0.11	0.31	47.38	28	94	6	279	106	8.10
	NFT	3.07	0.39	1.62	0.35	0.32	0.09	0.33	48.07	40	92	8	283	78	17.70
	Mean	3.20	0.41	1.66	0.37	0.32	0.10	0.31	47.51	34.00	88.25	8.25	286.25	89.75	10.60
Hydrofeed®	Sand	3.47	0.43	1.84	0.37	0.24	0.1	0.31	46.95	38	79	6	401	109	15.10
	Gravel	3.42	0.55	1.82	0.63	0.21	0.14	0.3	46.74	47	83	8	282	91	25.70
	Perlite	3.45	0.43	1.78	0.51	0.21	0.14	0.31	47.48	33	86	6	306	100	17.90
	NFT	3.39	0.5	1.93	0.41	0.24	0.16	0.3	47.19	41	81	9	274	81	20.00
	Mean	3.43	0.48	1.84	0.48	0.23	0.14	0.31	47.09	39.75	82.25	7.25	315.75	95.25	19.68
Trial Mean		**3.25**	**0.41**	**1.83**	**0.47**	**0.25**	**0.12**	**0.29**	**46.98**	**36.21**	**84.32**	**9.93**	**343.11**	**114.36**	**15.32**

5.3.5 Rooting performance in response to different fertilisers, clones and substrates at the third harvest

The third and final harvest from the hydroponic stock plants taken on 14/01/2002, proved to be the most successful in terms of rooting performance (Table 5.7). Prior to determining the rooting interaction to nutrient concentrations, the effects of cutting dimensions on rooting were examined utilising linear regression tests. Cutting length (cm), root collar diameter (mm),

sturdiness ratio (see Chapter 4) and biomass index were all correlated with rooting to identify if any linear relationship existed.

Table 5.7 Rooting results and dimensions of third harvest - 28/03/2002

Fertiliser	Substrate	% rooted	RCD(mm)	Length (cm)
Hydroponica®	Gravel	65.00	1.97	5.27
Hydroponica®	Sand	62.90	2.02	5.41
Hydroponica®	Perlite	47.20	2.06	5.21
Hydroponica ®	NFT	57.50	2.04	5.69
Murashige & Skoog	Gravel	57.10	1.71	5.29
Murashige & Skoog	Sand	37.60	2.09	5.23
Murashige & Skoog	Perlite	62.10	1.72	5.42
Murashige & Skoog	NFT	57.40	1.66	5.01
Natgro ®	Gravel	51.50	1.98	5.20
Natgro ®	Sand	56.40	1.93	5.67
Natgro ®	Perlite	64.40	2.03	5.43
Natgro ®	NFT	59.30	1.77	4.91
Hoaglands	Gravel	28.10	1.86	4.77
Hoaglands	Sand	38.00	1.49	4.33
Hoaglands	Perlite	57.10	2.00	5.29
Hoaglands	NFT	34.20	1.60	4.46
Mondi Orange	Gravel	41.30	1.88	5.10
Mondi Orange	Sand	60.80	1.81	5.40
Mondi Orange	Perlite	46.10	1.96	4.96
Mondi Orange	NFT	39.30	1.57	4.90
Mondi MM4	Gravel	61.20	2.02	5.56
Mondi MM4	Sand	45.90	1.92	4.99
Mondi MM4	Perlite	47.90	1.99	5.42
Mondi MM4	NFT	54.00	1.71	5.22
Hydrofeed ®	Gravel	40.90	2.10	4.94
Hydrofeed ®	Sand	33.30	1.92	4.51
Hydrofeed ®	Perlite	47.80	2.23	5.95
Hydrofeed ®	NFT	26.80	2.10	5.30

The biomass index is a derived value calculated as: BI = RCD^2 x Ht. Where BI = Biomass index, RCD = root collar diameter and Ht = length of stem. This index is used in weed competition and coppice efficiency trials to determine the growth response of plantation trees to weed effects (Dr. K. LITTLE, pers. comm., 2002).

Rooting showed a poor correlation with the biomass index (r = 0.227). However, there were significant differences (5% level) within treatments for the biomass index. Root collar diameter showed a very weak correlation with rooting and there were no significant differences in RCD values (Table 5.8). The sturdiness ratio did not correlate with rooting, but, there were significant differences (5% level) within the data set values. The length of cuttings had the best correlation (r = 0.227) with rooting with the model accounting for 22.40% of the

variance. There was also significant variation in cuttings length (1% level). A step-wise regression model confirmed that only cutting length was weakly responsive to rooting and accounted for 22.4% of the variance as compared to 20.8% for all explanatory variables. However, ANOVA tests (one-way, no blocking) showed that length of cutting did not significantly differ at either the clone, substrate, or fertiliser levels.

Table 5.8 Linear regression results for interaction of cutting dimensions with rooting

	Biomass index	Length (cm)	RCD (mm)	Sturdiness ratio
Rooting	0.227	0.483	0.142	0.223
F. pr.	*	**	n.s.	*
Std error	0.313	3.400	7.570	5.420
% variance	4.00	22.40	0.80	3.8

n.s. = not significant; * = 5%; ** = 1%; (d.f. = 81)

The third harvest produced an overall rooting mean of 48.6%. This was a dramatic improvement over the last two harvests and it would appear that the hydroponic hedge productivity has improved with age and with stronger rooting systems. Whether lighting is in fact a limiting factor remains an unresolved issue. A test for significant differences for rooting between the three clones (ANOVA test, one way, no blocking; d.f. = 82) showed that rooting differed (1% level) at the clone level and the order of rooting had not changed from the first harvest: 1). NH000 - 60.8% 2). GN156 - 45.1% 3). GN107 - 42.1%.

There were significant differences at the fertiliser level (5%) and the order of ranking for fertiliser was:

1	Natgro®	57.9%
2	Hydroponica®	57.7%
3	Murashige & Skoog	53.6%
4	Mondi MM4	52.3%
5	Mondi Orange	46.9%
6	Hoaglands	39.4%
7	Hydrofeed®	37.2%

There were no significant differences at the substrate level and it could be rejected as a source of variance. This trend confirms the results from the first harvest and the previous experiments which showed that differences in rooting were strongly related to the clonal effect and weakly responsive to different fertilisers. The results of rooting in response to

fertiliser and substrate showed the worst results to be from a combination of Hydrofeed® and NFT, whilst the best results were from a Hydroponica® and gravel combination (Table 5. 9).

Table 5.9 Matrix of rooting results by fertiliser and substrate - third harvest 28/03/2002

	Hydroponica®	Murashige & Skoog	Natgro®	Hoaglands	Mondi Orange	Mondi MM4	Hydrofeed®
Gravel	**65.00****	57.10	51.50	28.10	41.30	61.20	40.90
Sand	62.90	37.60	56.40	38.00	60.80	45.90	33.30
Perlite	47.20	62.10	64.40	57.10	46.10	47.90	47.80
NFT	**57.50 c**	57.40	59.30	34.20	39.30	54.00	**26.80***

* = worst rooting result; ** = best rooting result; c= control

5.3.6 Effect of nutrient concentration on rooting performance at third harvest

The concept of element ratios playing a role in plant nutrition had not been considered up until this stage. Several authors, including CROMER *et al.* (1981), SCHÖNAU and HERBERT (1989) and HERBERT (1996) allude to its importance, especially the ratios Ca:Mg, N:P, N:K, and P:K. In this experiment, ratios between these elements were included to help explain the interaction of rooting within the nutrient groups (Table 5.10).

Table 5.10 Plant tissue analysis across all treatments - third harvest

Nutrient	media	N %	P %	K %	Ca %	Mg %	Na %	B mg/kg	Zn mg/kg	Cu mg/kg	Mn mg/kg	Fe mg/kg	Root %	N:P	N:K	P:K	Ca:Mg
Hydroponica®	Sand	2.12	0.35	2.01	0.82	0.24	0.14	93.00	56.00	12.00	590.0	130.0	62.90	0.17	0.95	0.17	3.42
Hydroponica®	Gravel	2.28	0.34	2.12	0.71	0.26	0.14	112.00	58.00	15.00	401.0	99.0	65.00	0.15	0.93	0.16	2.73
Hydroponica®	Perlite	2.39	0.34	2.08	0.87	0.28	0.15	94.00	61.00	12.00	403.0	151.0	47.20	0.14	0.87	0.16	3.11
Hydroponica®	NFT	2.24	0.31	2.12	0.69	0.27	0.18	139.00	53.00	13.00	274.0	90.0	57.50	0.14	0.95	0.15	2.56
mean		**2.26**	**0.34**	**2.08**	**0.77**	**0.26**	**0.15**	**109.50**	**57.00**	**13.00**	**417.0**	**117.5**	**57.70**	**0.15**	**0.92**	**0.16**	**2.95**
M & S	Sand	2.78	0.33	2.01	0.62	0.20	0:15	40.00	107.00	19.00	1856.0	388.0	37.60	0.12	0.72	0.16	3.10
M & S	Gravel	2.86	0.32	2.05	0.67	0.20	0.13	51.00	128.00	19.00	984.0	167.0	57.10	0.11	0.72	0.16	3.35
M & S	Perlite	2.81	0.33	1.88	0.68	0.22	0.16	38.00	115.00	17.00	1176.0	155.0	62.10	0.12	0.67	0.18	3.09
M & S	NFT	2.69	0.30	2.15	0.61	0.24	0.19	54.00	98.00	17.00	625.0	384.0	57.40	0.11	0.80	0.14	2.54
mean		**2.79**	**0.32**	**2.02**	**0.65**	**0.22**	**0.16**	**45.75**	**112.00**	**18.00**	**1160.3**	**273.5**	**53.60**	**0.11**	**0.73**	**0.16**	**3.02**
Natgro®	Sand	2.41	0.32	1.68	0.78	0.30	0.16	54.00	45.00	8.00	372.0	109.0	56.40	0.13	0.70	0.19	2.60
Natgro®	Gravel	2.39	0.33	1.57	0.84	0.32	0.15	57.00	48.00	13.00	374.0	109.0	51.50	0.14	0.66	0.21	2.63
Natgro®	Perlite	2.32	0.32	1.67	0.79	0.33	0.15	56.00	46.00	8.00	297.0	93.0	64.40	0.14	0.72	0.19	2.39
Natgro®	NFT	2.06	0.26	1.85	0.67	0.32	0.17	107.00	48.00	8.00	187.0	76.0	59.30	0.13	0.90	0.14	2.09
mean		**2.29**	**0.31**	**1.69**	**0.77**	**0.32**	**0.16**	**68.50**	**46.75**	**9.25**	**307.5**	**96.8**	**57.90**	**0.13**	**0.74**	**0.18**	**2.43**
Hoaglands	Sand	2.43	0.32	1.81	0.96	0.29	0.15	38.00	59.00	11.00	585.0	106.0	38.00	0.13	0.74	0.18	3.31
Hoaglands	Gravel	2.53	0.32	1.94	0.86	0.24	0.11	39.00	57.00	10.00	357.0	76.0	28.10	0.13	0.77	0.16	3.58
Hoaglands	Perlite	2.41	0.34	1.83	0.93	0.30	0.12	41.00	68.00	9.00	455.0	81.0	57.10	0.14	0.76	0.19	3.10
Hoaglands	NFT	2.46	0.30	2.25	0.92	0.36	0.20	62.00	54.00	9.00	384.0	71.0	34.20	0.12	0.91	0.13	2.56
mean		**2.46**	**0.32**	**1.96**	**0.92**	**0.30**	**0.14**	**45.00**	**59.50**	**9.75**	**445.3**	**83.5**	**37.20**	**0.13**	**0.80**	**0.17**	**3.14**
Mondi Orange	Sand	2.32	0.35	1.44	0.82	0.26	0.15	56.00	69.00	11.00	791.0	92.0	60.80	0.15	0.62	0.24	3.15
Mondi Orange	Gravel	1.80	0.45	1.34	1.09	0.30	0.16	73.00	78.00	9.00	410.0	99.0	41.30	0.25	0.74	0.34	3.63
Mondi Orange	Perlite	2.42	0.35	1.39	0.93	0.27	0.15	56.00	84.00	10.00	368.0	121.0	46.10	0.14	0.57	0.25	3.44
Mondi Orange	NFT	1.77	0.53	1.47	0.84	0.35	0.14	97.00	76.00	6.00	281.0	109.0	39.30	0.30	0.83	0.36	2.40
mean		**2.08**	**0.42**	**1.41**	**0.92**	**0.29**	**0.15**	**70.50**	**76.75**	**9.00**	**462.5**	**105.3**	**46.90**	**0.21**	**0.69**	**0.30**	**3.16**
Mondi MM4	Sand	1.90	0.29	1.26	0.77	0.35	0.12	47.00	54.00	9.00	411.0	82.0	45.90	0.15	0.66	0.23	2.20
Mondi MM4	Gravel	2.27	0.32	1.26	0.83	0.37	0.14	58.00	56.00	11.00	220.0	107.0	61.20	0.14	0.56	0.25	2.24
Mondi MM4	Perlite	2.30	0.33	1.23	0.76	0.35	0.11	49.00	60.00	9.00	251.0	103.0	47.90	0.14	0.53	0.27	2.17
Mondi MM4	NFT	1.88	0.30	1.25	0.59	0.33	0.12	70.00	48.00	8.00	108.0	79.0	54.00	0.16	0.66	0.24	1.79
mean		**2.09**	**0.31**	**1.25**	**0.74**	**0.35**	**0.12**	**56.00**	**54.50**	**9.25**	**247.5**	**92.8**	**52.30**	**0.15**	**0.60**	**0.25**	**2.10**
Hydrofeed®	Sand	2.19	0.33	1.65	1.12	0.25	0.16	62.00	56.00	10.00	752.0	148.0	33.30	0.15	0.75	0.20	4.48
Hydrofeed®	Gravel	2.29	0.34	1.53	1.20	0.28	0.17	66.00	64.00	8.00	352.0	142.0	40.90	0.15	0.67	0.22	4.29
Hydrofeed®	Perlite	2.35	0.31	1.48	1.12	0.25	0.17	51.00	58.00	8.00	356.0	121.0	47.80	0.13	0.63	0.21	4.48
Hydrofeed®	NFT	2.11	0.27	1.54	0.72	0.24	0.04	75.00	55.00	6.00	167.0	75.0	26.80	0.13	0.73	0.18	3.00
mean		**2.23**	**0.31**	**1.55**	**1.04**	**0.26**	**0.14**	**63.50**	**58.25**	**8.00**	**406.8**	**121.5**	**37.20**	**0.14**	**0.70**	**0.20**	**4.06**

Using analysis of variance, the effect of nutrient differences were sought at the fertiliser and substrate levels. Juvenile coppice material sent for dry mass analysis was grouped by substrate and fertiliser (d.f. = 82), with different clones being pooled within the blocks and plots (Table 5.11).

Table 5.11 ANOVA of foliar nutrient differences at the fertiliser and substrate level

	N %	P %	N:P %	N:K %	K %	P:K %	Ca %	Ca:Mg %	Mg %	Na %	B mg/kg	Cu mg/kg	Fe mg/kg	Zn mg/kg	Mn mg/kg
Fertiliser	**	**	**	**	**	**	**	**	**	n.s.	**	**	**	**	**
Substrate	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

n.s. = not significant; * = 5% ; ** = 1%

There were significant differences (1%) at the fertiliser level for all elements, except sodium. Hydroponica® accounted for differences in boron and nitrogen : potassium ratio, whilst Mondi Orange solution was responsible for significant elemental differences in phosphorus, phosphorus : potassium and nitrogen : phosphorus ratios. Mondi MM4 accounted for differences in potassium. Murashige and Skoog (1962) accounted for differences in calcium, copper, iron, magnesium, nitrogen, manganese and zinc, whilst Hydrofeed® accounted for the calcium : magnesium ratio differences. As expected, there were no significant differences in elemental concentrations at the substrate level.

A further ANOVA test (one-way, no blocking) to determine rooting differences at the fertiliser level, showed a significant difference (F. pr. 0.023). This was in contrast to the first harvest where there were no differences amongst solution types. It may indicate that ramet nutrient uptake is changing as plants age physiologically or become accustomed to their specific nutrient feed.

A multiple linear regression model for the relationship between rooting and nutrients, and nutrient ratios accounted for **47.4%** (d.f. = 82) of the variance with a standard error of **8.13**. No specific element or ratio correlated even moderately with rooting.

For the same data set, the model from a step-wise regression contained a very interesting output that accounted for **51.7%** of the variance, the highest ever achieved in these series of experiments, whilst the standard error dropped to **7.80**. The element variables identified as interacting with rooting included calcium, manganese, nitrogen, sodium, phosphorus, nitrogen : potassium ratio, nitrogen : phosphorus ratio, phosphorus : potassium ratio and zinc. The resultant equation was: **rooting% = 546 - 68.8(Ca) - 0.01834(Mn) - 167.2(N) + 279.9(Na) - 234.7(N:K) - 1343(N:P) + 1262(P) - 771(P:K) + 0.341(Zn).**

The step-wise regression was recalculated with only individual element values included (all the ratio values removed) and the output identified only calcium and sodium as interacting with rooting. However, it could only account for **25.7%** of the variance whilst the standard error had risen to **9.67**. The resultant equation was: **Rooting% = 60.6 - 36.3(Ca) + 126.4(Na).**

5.3.7 Rooting responses to consecutive harvests

A comparison over all three cutting assessments produced an overall rooting mean of 25.33%. The mean cutting length was 4.36 cm and the mean root collar diameter 1.56 mm.

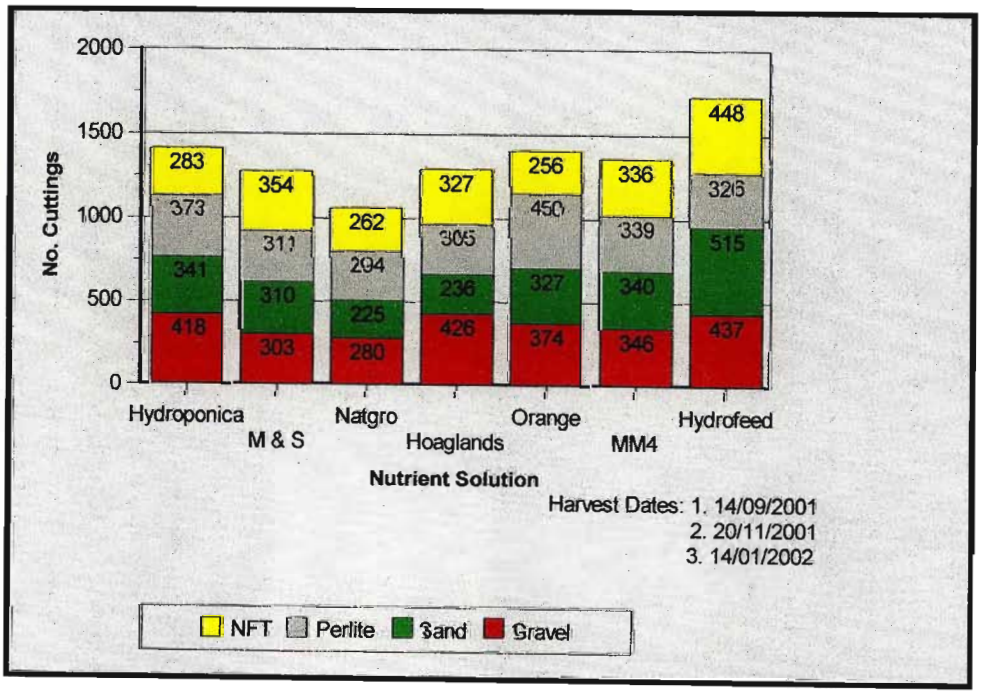
Mean rooting correlated weakly with cutting length (r = 0.491; d.f. = 250) and even poorer with RCD (r = 0.487). However, estimates of the regression coefficients (t-test values) did indicate that there were significant differences over all three harvests for cutting length and RCD.

In a regression analysis, with both RCD and length as explanatory variates and rooting the response variate, the model could only account for 25.7% of the variance for a standard error of 19.2. A further step-wise regression to identify the dominant variate produced the same output with the same model variance and error values. The best linear equation to explain rooting was calculated as: **Root % = -8.84 + 4.12 (length) +10.42 (RCD).** Due to the low values of the model variance accountability (25.7%) and the weak correlation values, it would be safer not to estimate rooting performance as a function of RCD or cutting length.

5.3.7.1 Cutting yield from three consecutive harvests

At placement, a comparison of cutting yield by fertiliser type revealed that Hydrofeed® produced most cuttings overall, whilst Natgro ® produced the least (Figure 5.5).

Figure 5.5 Cuttings yield from three consecutive harvests



T-tests showed no differences between substrates for cutting yield. A summary of cuttings yield at the substrate level is shown in Table 5.12.

Table 5.12 Summary statistics for cutting yield by substrate

Summary statistic	Gravel	NFT	Perlite	Sand
Mean	369.14	323.71	342.57	327.71
Median	374.00	327.00	326.00	327.00
Minimum	280.00	256.00	294.00	225.00
Maximum	437.00	448.00	450.00	515.00
Range	157.00	192.00	156.00	290.00
Standard deviation	62.06	66.56	54.04	95.46
Standard error of mean	23.45	25.16	20.43	36.08
Coefficient of variation (CV%)	16.81	20.56	15.78	29.13
Skewness	-0.30	0.82	1.21	0.97

5.3.7.2 Overall rooting responses

A comparison over the three separate harvests showed that rooting improved dramatically at the third harvest (Table 5.13). The first two sets of results were disappointing and may have been due to the overly juvenile state of the hedge ramets.

Table 5.13 Summary statistics for mean rooting over three harvests

Summary statistic	Harvest 1	Harvest 2	Harvest 3
Mean	15.32	12.08	48.60

ANOVA tests (one-way, no blocking) to check for significant differences between clones, substrates, fertilisers and their interactions is summarised in Table 5.14.

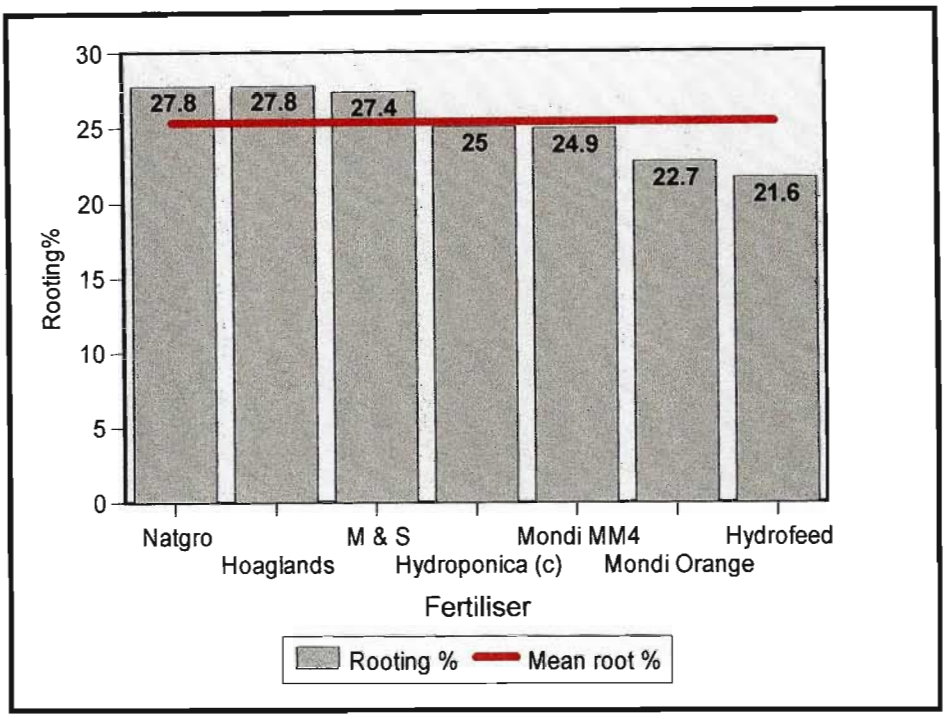
Table 5.14 Summary ANOVA of rooting differences within fertiliser, substrate and clone

	Clone ID	Fertiliser	Substrate	Fertiliser x Substrate	Substrate x Clone	Fertiliser x Clone
Root 1	**	*	n.s.	n.s.	n.s.	n.s.
Root 2	**	n.s.	n.s.	n.s.	n.s.	n.s.
Root 3	**	n.s.	n.s.	n.s.	n.s.	n.s.

n.s. = not significant; * = 5% ; ** = 1%; d.f. = 83

The overall mean rooting for the three test clones, over three harvests was 25.33%. The order of ranking was: (1) NH000 - 33.2% (2) GN156 - 21.8% and (3) GN107 - 21.0%. For all three sets of rooting data, there were significant differences between clones (Table 5.14). However, the ranking never altered and was always of the order NH000 > GN156 > GN107. Combined rooting results showed no significant differences at the fertiliser level (Figure 5.6).

Figure 5.6 Combined rooting results at the fertiliser level



There was a difference between fertilisers and their effect on rooting (5% level) at the first harvest. However, there were no further significant differences at the second and third harvests and it is unlikely that subsequent harvest results would respond differently.

There were no significant differences amongst the substrates over the three harvests (F. pr. 0.784; d.f. = 251) and the order of ranking was (1) perlite - 27.2% (2) gravel - 26.2% (3) NFT - 24.4% and (4) sand - 23.5%. On the basis of the data at hand (Table 5.14), it can be conclusively stated that overall differences in rooting performance were as a result of clonal differences and not due to fertiliser or substrate.

5.3.7.3 Rooting response to fertiliser and substrate interaction

Overall rooting responses were clearly as a result of clonal effects and not due to fertiliser and substrate combinations. Rooting increased from 15.32% in the first harvest to 48.60% in the third harvest, a 3.17 fold improvement (Table 5.15 and Figure 5.7). At the first harvest, the best rooting results were a combination of Hoaglands fertiliser and sand media (mean rooting = 30.30%). The second harvest showed a combination of Hoaglands and NFT (mean rooting = 33.06%) to yield the best result whilst from the last harvest, Hydroponica® combined with gravel was the best performer (mean = 65.00%) (Table 5.10 and Figure 5.7). At each harvest the control plot (Hydroponica® + NFT) had a lower mean rooting. On the basis of the

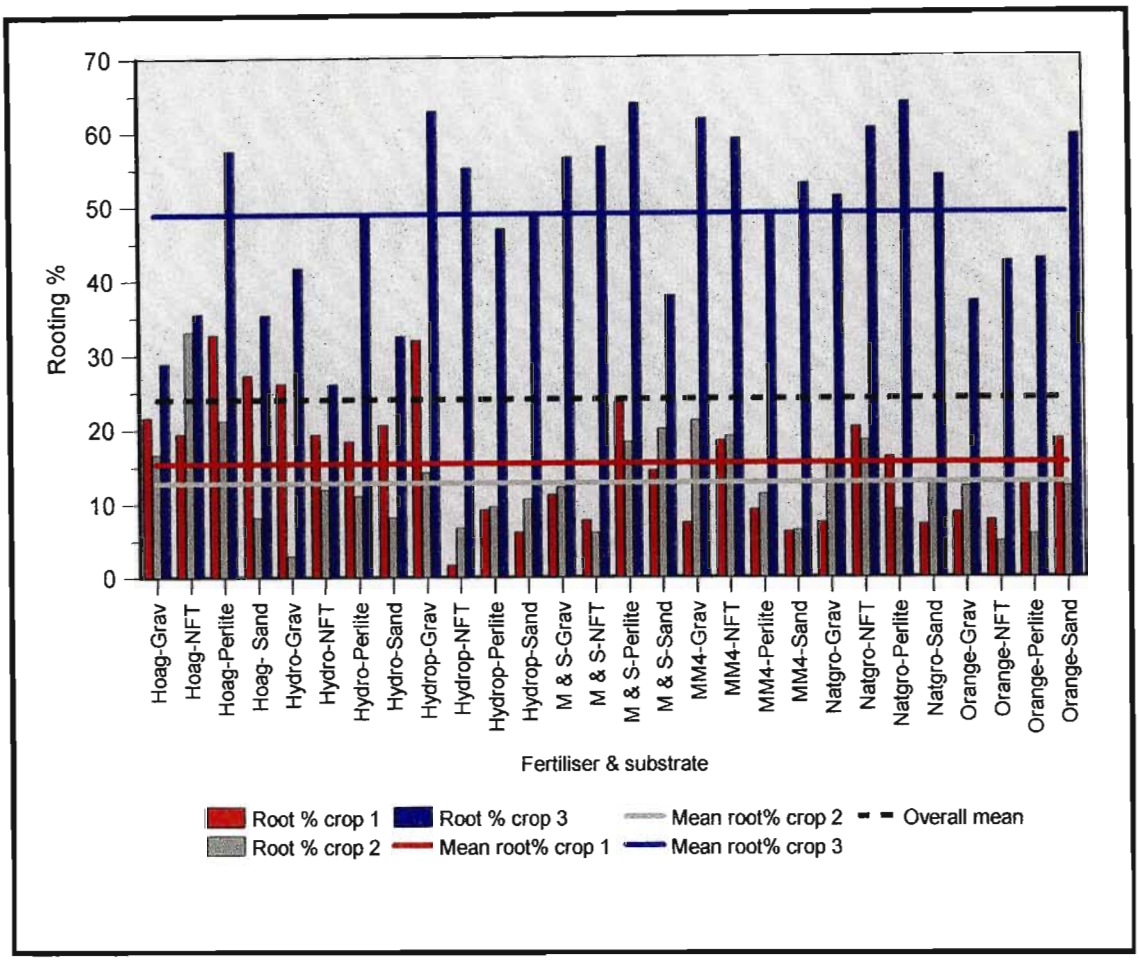
third and best set of rooting results, the recommended combination for the propagation of the three test clones would be Hydroponica® fertiliser combined with gravel.

Table 5.15 Comparison of rooting performance over three consecutive harvests

Fertiliser	Substrate	Root % 1	Root % 2	Root % 3	Overall Root %
Hoaglands	Gravel	23.60	16.67	28.10	22.79
Hoaglands	NFT	17.80	33.06	34.20	28.35
Hoaglands	Perlite	29.50	21.17	57.10	35.92
Hoaglands	Sand	30.30	8.18	38.00	25.49
Hoaglands		25.30	19.77	39.35	28.14
Hydrofeed®	Gravel	25.70	2.92	40.90	23.17
Hydrofeed®	NFT	20.00	11.93	26.80	19.58
Hydrofeed®	Perlite	17.90	11.02	47.80	25.57
Hydrofeed®	Sand	15.10	8.16	33.30	18.85
Hydrofeed®		19.68	8.51	37.20	21.79
Hydroponica®	Gravel	28.50	14.21	65.00	35.90
Hydroponica®	NFT (c)	1.00	6.80	57.50	21.77
Hydroponica®	Perlite	9.90	9.63	47.20	22.24
Hydroponica®	Sand	5.70	10.61	62.90	26.40
Hydroponica®		11.28	10.31	57.70	26.58
M & S	Gravel	10.50	12.21	57.10	26.60
M & S	NFT	9.20	6.07	57.40	24.22
M & S	Perlite	19.20	18.18	62.10	33.16
M & S	Sand	22.90	20.00	37.60	26.83
M & S		15.45	14.12	53.55	27.70
Mondi MM4	Gravel	7.70	21.08	61.20	29.99
Mondi MM4	NFT	17.70	18.98	54.00	30.23
Mondi MM4	Perlite	8.10	11.26	47.90	22.42
Mondi MM4	Sand	8.90	6.43	54.00	23.11
Mondi MM4		10.60	14.44	52.30	26.44
Natgro®	Gravel	7.60	15.38	51.50	24.83
Natgro®	NFT	19.80	18.40	59.30	32.50
Natgro®	Perlite	16.90	9.16	64.40	30.15
Natgro®	Sand	6.00	12.66	56.40	25.02
Natgro®		12.57	13.90	57.90	28.13
Mondi Orange	Gravel	8.80	12.17	41.30	20.76
Mondi Orange	NFT	9.00	4.88	39.30	17.73
Mondi Orange	Perlite	14.00	5.77	46.10	21.96
Mondi Orange	Sand	17.90	12.22	60.80	30.31
Mondi Orange		12.43	8.76	46.88	22.69

(c = control)

Figure 5.7 Consolidated rooting data over three harvests

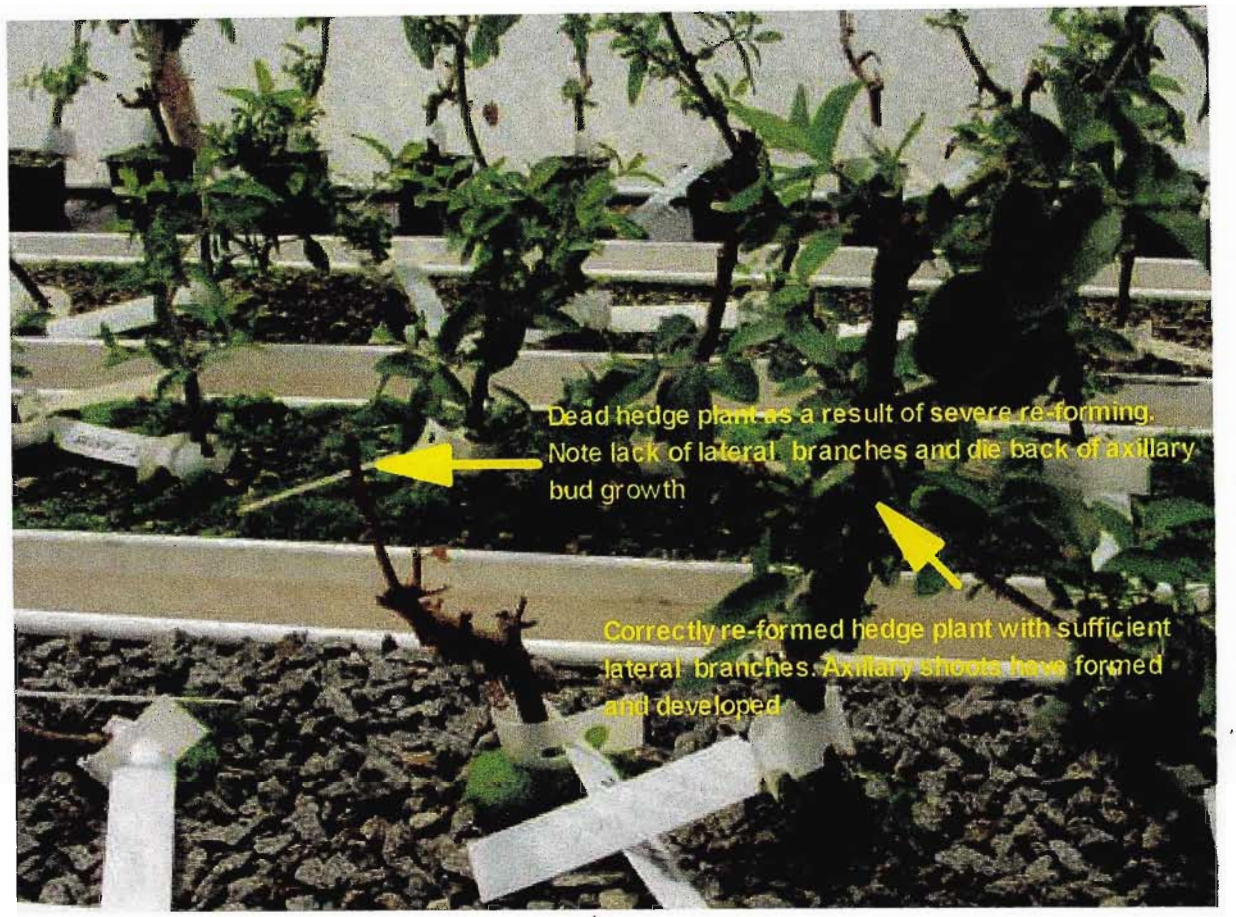


5.3.8 Ramet response to severe cutback

On 4/02/2002, at the end of the trial period, the plants in the seven hydroponic beds were cut back. Due to the removal of too many lateral branches, a 23.7% ramet mortality occurred across the entire experiment (Figure 5.8). It is imperative that every care be taken to ensure that not all lateral branches are cut back to the main stem as emerging axillary buds cannot sustain ramet survival alone. Analysis of variance tests showed that mortality was strongly linked to clonal differences (1% level; d.f. = 45) and mean mortality results were ranked as follows: (1) NH000 - 32.5% (2) GN107 - 17.0% and (3) GN156 - 16.9%.

Fertiliser (F. pr. 0.409) and substrate (F. pr. 0.221) levels did not affect ramet mortality and it can be conclusively stated that hedge plant mortality was associated with clonal differences. Clone NH000 consistently produced the best rooting results throughout the whole study yet was the most susceptible to plant mortality due to severe pruning.

Figure 5.8 Harvest techniques and their effect on hedge ramet survival



5.4 CONCLUSIONS

Temperature and relative humidity of the hydroponic growth room were highly variable. Temperature ranges of 2.46 °C to 37 °C and relative humidity extremes from 22.5% to 99% are of concern and must place hedges ramets under physiological stress. Major environmental fluctuations must be removed if the hedge plants are to perform optimally. Hydroponic hedges should be grown in climates with less extreme temperature fluctuations.

It has been observed by REUVENI *et al.* (1990) that in experiments with *E. camaldulensis*, a lower percentage of thin cuttings rooted than thick, non-lignified cuttings. From their observations, it is not recommended to use cuttings with a diameter of < 3 mm. The better rooting of thicker cuttings might be explained by their larger carbohydrate reserves. This influences rooting rate, and at later stages, the accelerated flushing and growth of the rooted cuttings. EDE *et al.* (1997) in their study of *Paulownia tomentosa* and *P. fortunei* found that regenerative ability was closely coupled with cutting diameter as cuttings from both species, that were 5 mm or less in diameter, produced less shoot growth. The largest cuttings produced the most vigorous shoot and root development. HENRY *et al.* (1992) found in their

study of Eastern Red Cedar (*Juniperus virginina* L.) that cutting length affected rooting, with root count and length highest in 25 cm long cuttings. NEWTON *et al.* (1996) state that rooting ability is often correlated with cutting length, reflecting the importance of stem volume for carbohydrate storage. Leaf morphology may have an effect on photosynthetic capacity, which is known to influence rooting ability through its effect on the carbon economy of a cutting. AIMERS-HALLIDAY *et al.* (1999) reported that the rooting of *E. grandis x E. nitens* clones is very variable. There appears to be no clear cut trends in the time it takes roots to emerge or in the type of cutting that roots best. In any single clone, some cuttings rooted within six weeks whilst others had only just formed roots six months after setting. Well developed root systems, with up to six roots emerging from callus could be found in both small and large diameter cuttings (2 to 8 mm RCD).

A comparison of cuttings dimensions from three harvests produced a mean cutting length of 4.4 cm and a mean RCD of 1.6 mm. Cutting dimensions did not correlate with rooting, although there were significant differences in cutting length and RCD. Variation in cutting length was as a result of selection of material on the part of the clonal team and can be corrected by continual training. The variation in RCD is not easy to estimate but can be monitored at harvest time and through random tests with a digital vernier calliper. The inclusion of the sturdiness ratio and the biomass index, both derived values, in the correlation with rooting response produced no proof of a linear relationship and are of no practical value at this stage.

Over the three harvests, the highest number of cuttings was yielded by Hydrofeed® fertiliser and the lowest by Natgro ®, although the overall difference was not significant. A high cutting yield from vigorous coppice growth does not necessarily correlate with a high rooting response and the rooting may decrease in hedges fertigated too frequently at high EC levels, especially with high nitrogen levels. DE ASSIS (2001) states that a moderate deficiency of nitrogen can improve rooting, but at higher concentration levels more energy is required for vegetative growth and especially leaf expansion. Consequently, carbohydrates are not stored at suitable levels resulting in a reduced C:N ratio. An extreme nitrogen deficiency can reduce rooting since it is necessary for nucleic acid and protein synthesis.

HERBERT (1996) has found that increased nutrient uptake following fertilizer application does not necessarily indicate better growth, nor does a decrease in nutrient concentration result in poorer growth. The best growth is achieved when there is an overall balance of nutrients, i.e. where nutrient ratios all approach their optima. However, some ratios are more

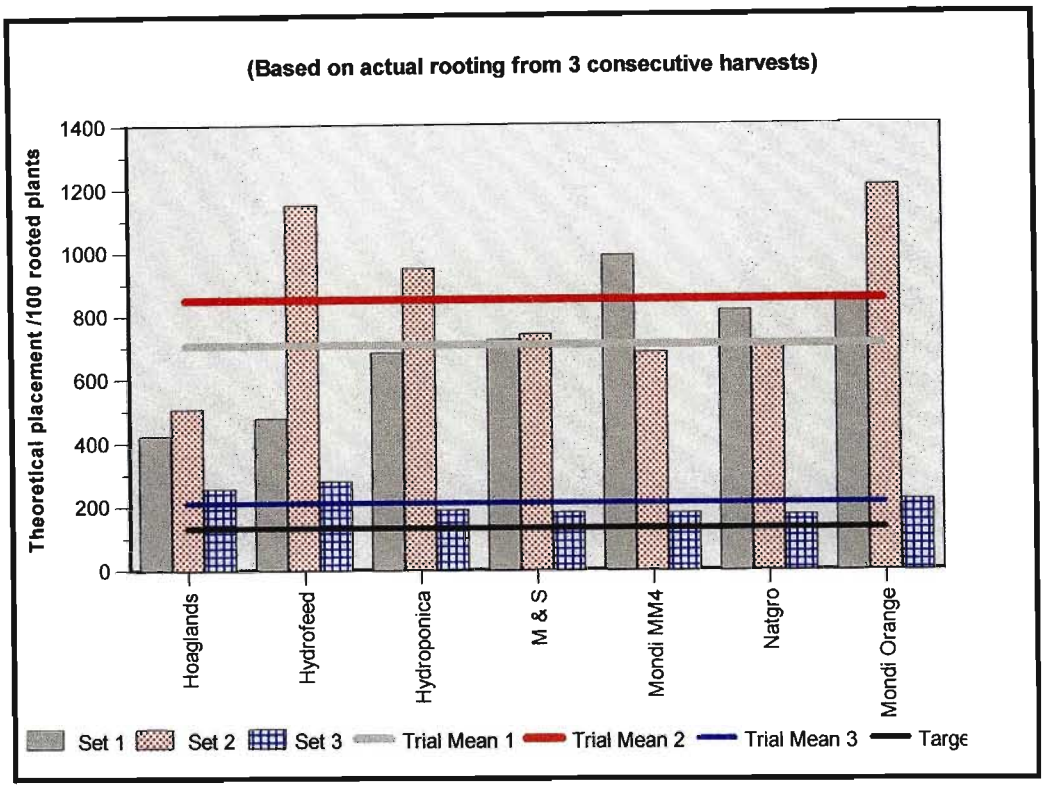
important than others and if the ratios of nitrogen : phosphorus, nitrogen : potassium, nitrogen : sulphur, phosphorus : potassium and calcium : magnesium are close to their optima, it is unlikely that growth rate can be increased through fertilising. It is possible that the optimum nutrient requirements for the production of wood fibre in a plantation and those required for the production of premium coppice material (juvenile state) in a hydroponic hedge plant may vary somewhat in terms of the different element concentrations.

Rooting performance responded strongly to genotype differences, whilst substrate and the interaction of clone by substrate, and clone by fertiliser did not significantly impact on rooting. This confirmed findings from the previous experiments that showed rooting responses to be strongly linked to clonal differences and weakly responsive to different fertilisers. However, by the third harvest, rooting differences were becoming more significant (F. pr. 0.023) in response to fertiliser type and this change may indicate that ramet nutrient uptake was changing as plants physiologically aged or became more accustomed to a specific nutrient feed.

The poor rooting results (mean = 15.3%) of the first harvest were similar to those experienced from first harvests in clonal field hedges. However, the results were somewhat perplexing in comparison to the good results achieved in the previous two experiments carried out under artificial lighting. Whereas it was first thought that light intensity levels of 3000-4000 Lux were insufficient to sustain a hydroponic ramet, it may be more correct to suggest that the normal insolation experienced in field conditions is too high (or not optimal) for inducing better rooting from coppice material. However, the better rooting results achieved at the third harvest tend to counteract this argument and the role of light and its effect on rooting requires further investigation.

It was reassuring to note the major improvement in rooting of the third harvest in comparison to the previous harvests. The rooting productivity of the hedge ramets improved 3.16 fold with age and with the development of more vigorous root systems. The physiological 'maturing' of the ramets may have had a beneficial effect on the active deployment of plant growth regulators involved in root initiation. Experience has shown that the first two to three coppice collections from any hydroponically sustained eucalypt hedge plant will result in erratic and poor rooting results, but will improve thereafter and from the evidence at hand, the target rooting figure of 75% for the *E. grandis x E. nitens* hybrid may become more attainable (Figure 5.9).

Figure 5.9 Theoretical cutting placement/100 rooted plants produced



An important objective of this experiment was to identify those nutrients most strongly associated with rooting in hybrid eucalypts. Dry mass tissue analysis results for the first and third harvests showed significant differences (at 1% level) for all elements at the fertiliser level, except for boron in the first harvest and sodium in the third harvest. In the first harvest, no specific fertiliser type accounted for all of the difference in nutrient concentrations. However, in the third harvest Murashige and Skoog (1962) nutrient feed accounted for differences in seven of the major nutrients.

At the third harvest assessment, the concept of element ratios playing a role in plant nutrition was realised. CROMER *et al.* (1981) suggested an optimum nitrogen : phosphorus ratio of 15 for *E. globulus* and *E. sieberi* whilst SCHÖNAU and HERBERT (1989) in their analysis of *E. grandis* trials in South Africa set the optimum at 13. Although the ratios of nitrogen : potassium, nitrogen : sulphur, phosphorus : potassium and calcium : magnesium may be useful, responses to fertilisers based on these ratios are complex. These types of interaction can be better assessed by designing element-exclusion experiments. A step-wise regression of the third harvest data identified the elements calcium, manganese, nitrogen, phosphorus, sodium, and zinc and the ratios nitrogen : potassium, nitrogen : phosphorus, phosphorus : potassium as responding to rooting. The inclusion of nutrient ratios has revealed an exciting direction in the identification of rooting response to nutrition. In the first harvest with its poor

rooting results, only one element (calcium) was identified whilst in the third harvest the inclusion of several element ratios has greatly improved the accuracy through which we can predict rooting response to nutrient concentrations.

Element concentrations appeared to be changing concentration direction relative to one another and this 'push-pull' effect may be the catalyst that stimulates rooting. An increase in one element, or group of elements, may cause a decrease in another element, or group of elements, with a resultant effect on rooting. The inverse correlation (eight out of fifteen elements) of a number of elements with rooting strongly alludes to competitive effects amongst nutrients.

Many elements are thought to be active in root induction, including nitrogen, phosphorus, calcium, magnesium, manganese, boron and zinc. Zinc, manganese and boron are known to influence the metabolic status of indole-3-acetic acid (IAA) (SVENSON *et al.*, 1995). According to ANDERSON (1986), the effects of micro-nutrients on rooting are variable. Manganese, due to its inhibitory effect, should be supplied at minimal concentrations, but boron and zinc are essential for the rooting process. Zinc increases the endogenous content of auxins by increasing tryptophan proportions while boron is involved in root initiation and root growth. From the step-wise regression model for harvest three, it can be confirmed that calcium, manganese, nitrogen, phosphorus and zinc also affect the rooting response of the *E. grandis x E. nitens* hybrid. Magnesium and boron were not identified as important and their exclusion cannot be explained at this stage.

CHAPTER 6

ROOTING RESPONSE OF EIGHT *EUCALYPTUS* COMMERCIAL CLONES TO AN EXPERIMENTAL RECIRCULATING HYDROPONIC SYSTEM

6.1 INTRODUCTION

FETT-NETO *et al.* (2001) describe adventitious rooting as an essential step in the vegetative propagation of economically important woody species. Adventitious rooting is a complex process, which is affected by multiple factors including phytohormones, phenolic compounds, nutritional status and genetic characteristics, as well as associated stress responses such as wounding, changes in plant water relations and loss of correlative whole plant influences.

SVENSON *et al.* (1995) note that studies have provided an understanding of the role of mineral elements in later root growth and development, but mineral elemental effects during the early developmental stages of adventitious root formation are less clear because of the difficulty in separating early endogenous root initiation from visible root growth and development.

Results from the previous three experiments (Chapters 3, 4 and 5) concur with the statement by SVENSON *et al.* (1995). Although certain elements appear to impact more on adventitious root initiation, the fluctuating results and the relatively low values of the regression models variance accountability (<51%) indicate that a number of parameters involved in rooting have not yet been identified and fall outside the realm of this study.

The objective of this experiment was to assess whether seven *Eucalyptus* hybrid clones (including three *E. grandis x E. nitens*, three *E. grandis x E. urophylla*, one *E. grandis x E. camaldulensis*) and one pure *E. grandis* could be sustained as hydroponic hedge plants in a commercial NFT unit. Specific objectives included: 1) assessing the effect of genotype on rooting differences (clonal effect) and 2) measuring changes in tissue dry mass analysis data.

6.2 MATERIALS AND METHODS

6.2.1 Systems design

The trial was planted on 18/6/2001 and all experimentation was carried out at the Mondi Forests Trahar Technology Centre, KwaZulu-Natal. One commercially available NFT recirculating unit was used (Figure 6.1). The unit consists of a modular plastic tubular (32 mm) frame, 1.43 m wide and 5.97 m long. Resting on the frame were eight 110 mm gutters, each planted with 40 stock plants. Plants were contained in 60 mL white round inserts.

The hydroponic unit was placed in a 8 x 30 m plastic covered tunnel with 40% shadecloth covering. Light readings in the tunnel exceeded 10000 Lux on a clear day. Extractor fans were set to activate at 28 ^{0}C. No wet wall was available.

Figure 6.1 Testing of eight eucalypt genotypes in a commercial NFT unit

6.2.2 Experimental design and layout

The trial was established as a split plot design. There were no substrates (NFT only) and each clone (eight eucalypt clones tested) occupied a whole gutter (plot). Each plot contained 40 ramets of a clone, giving a total of 320 plants.

6.2.3 Elemental composition of test solution

A solution of Hydroponica® and calcium nitrate was applied as the nutrient feed. The solution strength was maintained at 1100-1200 µS/cm and a pH of 5.8 to 6.7. Solution samples were submitted to the ARC in Nelspruit for chemical analysis (Table 6.1).

Table 6.1 Analysis of solution at start of a cycle - 06/02/2002

	pH	EC S/cm	TDS mg/l	Ca mg/l	Mg mg/l	K mg/l	Na mg/l	Cl^- mg/l	NO_3^- mg/l	NO_2 mg/l	SO_4^{2-} mg/l	Zn mg/l	Cu mg/l	Mn mg/l	Fe mg/l	B mg/l	NH_3 mg/l	P mg/l	N mg/l
Water	8.39	105	73.36	12.7	36.5	32	290	8.55	0-10	0.0	0.00	0.02	0.01	0.005	0.02	0.25	0.000	<0.05	<1
Solution 1	6.48	1381	966.7	60.8	35.6	197	375	14.0	100-150	5.0	<400	0.44	0.21	0.192	0.53	1.20	10-25	6.50	<1

Solution 1 = Hydroponica®

6.2.4 Cultural practices

The same protocols were applied as in the previous experiment. (See Chapter 4.2.6).

6.2.5 Preliminary measurements

From 18/6/2001 to 24/6/2002, a Hobo data logger was set to record whether the correlation between temperature and relative humidity continued to be inversely related as was recorded in the previous experiment. The results are discussed in Chapter 5.

6.2.6 Plant tissue analysis

All nutrient analyses were carried out by the Cedara Plant Laboratory. The technique that was used is summarised in Chapter 3.

6.2.7 Stem cuttings and harvest intervals

Stem cuttings were prepared as described in Chapter 3.2.13. The cutting lengths and root collar diameters (RCD) of all rooted plants were measured at a destructive harvest. Greenhouse protocols remained the same as those discussed in Chapter 5.2.8. Three consecutive coppice harvests were completed (14/09/2001, 31/10/2001, 21/01/2002). Cuttings were set in the greenhouse for 60 days and then moved to a plastic covered tunnel for a further 15 days prior to destructive sampling.

6.3 RESULTS AND DISCUSSION

6.3.1 Relationship of temperature and relative humidity in hydroponic tunnel

The recordings and findings are discussed in Chapter 5.3.1.

6.3.2 Effect of genotype on cuttings yield

Cuttings from the first placement (14/09/2001) had a mean of 117.1 cuttings/clone whilst from the second harvest (31/10/2001) a mean of 186 cuttings/clone was recorded. A t-test showed that the means were significantly different ($p = 0.015$). The third harvest resulted in a mean of 188.5 cutting/clone and there were no significant differences between harvest two and harvest three ($p = 0.940$). The lack of a noticeable change in cutting yield between harvest two and harvest three (Figure 6.2) may indicate a rapid maturing of the hedges because of the greater espacement between ramets as compared to the previous trials (Figure 6.3).

Figure 6.2 Cutting yield from three consecutive harvests

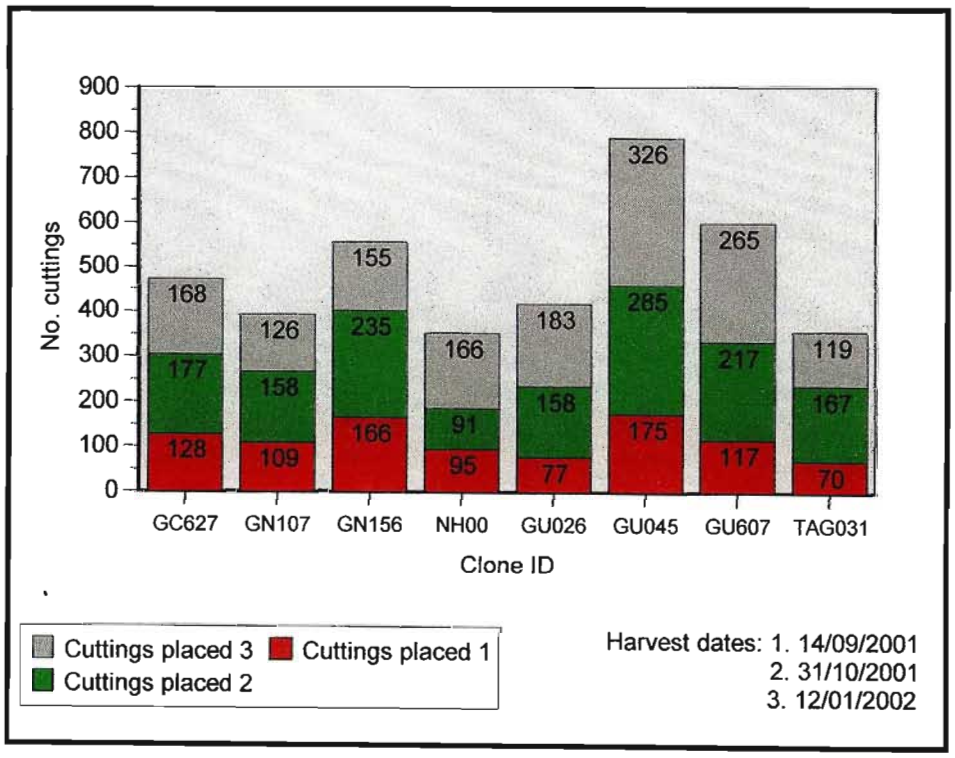


Figure 6.3 ***E. grandis x E. nitens*** **hybrid clone prior to second harvest**

6.3.3 Correlation of cutting dimensions and rooting performance

On 31/10/2001 and 12/1/2002, rooted cuttings were measured to determine length of cutting (cm), and root collar diameter (mm). The previous experiment (Chapter 5) showed no correlation of rooting with either the biomass or sturdiness ratio and these values were not included in the data (Table 6.2).

Table 6.2 Consolidated rooting data for experiment over three harvests

	No. replicates rooted harvest 1	No. replicates rooted harvest 2	No. replicates rooted harvest 3	Mean length cm harvest 2	Mean length cm harvest 3	Mean RCD mm harvest 2	Mean RCD mm harvest 3	Mean root% harvest 1	Mean root% harvest 2	Mean root% harvest 3
NH000	19	13	35	4.46	5.51	1.89	2.02	20.00	14.29	21.08
GN107	14	7	44	5.43	5.54	1.68	1.91	12.84	4.43	34.92
GN156	73	21	68	4.79	5.73	1.21	2.09	43.98	8.94	43.87
GU607	69	100	158	4.67	5.32	1.70	1.81	58.97	46.08	59.62
GU045	100	81	152	5.03	6.52	1.44	2.12	57.14	28.42	46.63
GU026	34	73	135	5.12	5.49	1.43	2.13	44.16	46.20	73.77
GC627	61	57	135	4.97	6.04	1.33	2.17	47.66	32.20	80.36
TAG31	42	58	43	4.39	4.93	1.46	1.80	60.00	34.73	36.13
Mean	51.5	51.25	96.25	4.83	5.75	1.50	2.03	46.36	29.41	54.08

For the combined data set, there were significant differences in cutting length and RCD between clones (F. pr <.001; d.f. =769). The grand rooting mean for the experiment was 39.85%, the mean rooted cutting length was 5.25 cm and the mean RCD was 1.76 mm. Rooting showed moderately weak correlations with cutting length (r = 0.436) and RCD (r = 0.511). Step-wise regression identified RCD as playing a stronger linear relationship with rooting with the equation: **rooting %= -23.2 + 34.9 (RCD)**. The model could only account for 20.9% of variance with a high standard error of 18.9. The regression estimate with both RCD and cutting length was less accurate and percentage variance dropped to 16.6 with a standard error increasing to 19.4. On the basis of this data, and the weak significance of the F-value (F. pr. 0.121), it would be correct to state that cutting length and RCD do not interact strongly with rooting and cannot accurately predict rooting in a model equation. This reinforces the findings from the previous experiment (Chapter 5).

A test for the effect of genotype on rooting performance showed a significant difference at the 5% level only (F. pr. 0.03.9) over the three harvests (Table 6.3).

Table 6.3 Effect of genotype on rooting results over three harvests

	NH000	GN107	GN156	GU026	GU045	GU607	GC627	TAG031
Mean rooting %	18.5	17.4	32.3	54.7	44.1	54.9	53.4	43.6

d.f. = 23; s.e.d = 12

6.3.4 Effect of nutrient concentration on rooting performance

Three sets of coppice material were submitted to Cedara Plant Laboratory for dry mass analysis (Table 6.4, Table 6.5 and Table 6.6).

Table 6.4 Plant tissue analysis across all clones - 14/09/2001

Clone I.D	N %	P %	K %	Ca %	Mg %	Na %	B mg/kg	Zn mg/kg	Cu mg/kg	Mn mg/kg	Cutting Placed	Cutting Rooted	Root %
GC627	2.46	0.35	1.78	0.49	0.29	0.12	86.00	222.00	13.00	195.00	128	61	47.66
Mean	2.46	0.35	1.78	0.49	0.29	0.12	86.00	222.00	13.00	195.00	128	61	47.66
GN107	2.73	0.45	1.53	0.30	0.20	0.17	99.00	106.00	13.00	368.00	109	14	12.84
GN156	2.41	0.47	1.84	0.34	0.23	0.12	108.00	112.00	13.00	363.00	166	73	43.98
NH000	2.12	0.39	1.52	0.36	0.25	0.13	71.00	86.00	11.00	387.00	95	19	20.00
Mean	2.42	0.44	1.63	0.33	0.23	0.14	92.67	101.33	12.33	372.67	123.33	35.33	28.65
GU026	3.21	0.45	1.93	0.42	0.32	0.16	122.00	167.00	16.00	295.00	77	34	44.16
GU045	2.33	0.46	2.46	0.58	0.43	0.16	99.00	140.00	13.00	224.00	175	100	57.14
GU607	3.33	0.43	2.06	0.48	0.33	0.14	76.00	128.00	15.00	283.00	117	69	58.97
Mean	2.91	0.45	2.15	0.49	0.36	0.15	99.00	145.00	14.67	267.33	123.00	67.67	55.01
TAG031	3.16	0.44	1.59	0.44	0.28	0.19	111.00	224.00	17.00	259.00	70	42	60.00
Mean	3.16	0.44	1.59	0.44	0.28	0.19	111.00	224.00	17.00	259.00	70	42	60.00
Trial Mean	**2.74**	**0.42**	**1.79**	**0.44**	**0.29**	**0.15**	**97.17**	**173.08**	**14.25**	**273.50**	**111.08**	**51.50**	**46.36**

Table 6.5 Plant tissue analysis across all clones - 31/10/2001

Clone ID	N %	P %	K %	Ca %	Mg %	Na %	B mg/kg	Zn mg/kg	Cu mg/kg	Mn mg/kg	Fe mg/kg	Cutting placed	Cutting rooted	Root %	RCD mm	Length cm
GC627	2.25	0.21	1.45	0.87	0.27	0.04	62.00	79.00	10.00	178.00	120.0	177	57	32.20	1.33	4.97
Mean	2.25	0.21	1.45	0.87	0.27	0.04	62.00	79.00	10.00	178.00	120.0	177.00	57.00	32.20	1.33	4.97
GN107	2.17	0.26	1.42	0.42	0.21	0.10	66.00	54.00	12.00	260.00	99.0	158	7	4.43	1.68	5.43
GN156	2.89	0.31	1.63	0.45	0.23	0.06	79.00	68.00	12.00	300.00	138.0	235	21	8.94	1.21	4.80
NH000	2.32	0.31	1.44	0.56	0.26	0.13	52.00	46.00	13.00	259.00	111.0	91	13	14.29	1.89	4.46
Mean	2.46	0.29	1.50	0.48	0.23	0.10	65.67	56.00	12.33	273.00	116.0	161.33	13.67	8.47	1.59	4.90
GU026	2.59	0.29	1.84	0.55	0.31	0.14	91.00	58.00	12.00	264.00	109.0	158	73	46.20	1.43	5.12
GU045	2.05	0.31	1.74	0.79	0.36	0.12	66.00	53.00	10.00	156.00	177.0	285	81	28.42	1.44	5.02
GU607	2.60	0.32	1.90	0.69	0.32	0.15	95.00	56.00	12.00	216.00	197.0	217	100	46.08	1.70	4.67
Mean	2.41	0.31	1.83	0.68	0.33	0.14	84.00	55.67	11.33	212.00	161.0	220.00	84.67	38.48	1.52	4.94
TAG31	2.03	0.24	1.31	0.69	0.31	0.13	67.00	100.00	12.00	162.00	125.0	167	58	34.73	1.46	4.39
Mean	2.03	0.24	1.31	0.69	0.31	0.13	67.00	100.00	12.00	162.00	125.0	167	58	34.73	1.46	4.39
Trial Mean	**2.29**	**0.26**	**1.52**	**0.68**	**0.28**	**0.10**	**69.75**	**72.75**	**11.42**	**206.25**	**130.5**	**181.33**	**53.33**	**29.41**	**1.48**	**4.80**

Table 6.6 Plant tissue analysis across all clones - 12/01/2002

Clone I.D	N %	P %	K %	Ca %	Mg %	Na %	B mg/kg	Zn mg/kg	Cu mg/kg	Mn mg/kg	Fe mg/kg	Cutting placed	Cutting rooted	Rooting %	RCD mm	Length cm
GC627	1.37	0.19	1.27	1.56	0.35	0.06	31.00	54.00	6.00	149.00	97.00	168	135	80.36	2.17	6.04
Mean	1.37	0.19	1.27	1.56	0.35	0.06	31.00	54.00	6.00	149.00	97.00	168.00	1350.00	80.36	2.17	6.04
GN107	1.38	0.23	1.27	0.70	0.26	0.14	40.00	40.00	6.00	191.00	82.00	126	44	34.92	1.91	5.54
GN156	1.63	0.27	1.47	0.86	0.30	0.11	43.00	60.00	6.00	225.00	74.00	155	68	43.87	2.09	5.73
NH000	1.22	0.22	1.30	0.67	0.34	0.14	35.00	35.00	6.00	157.00	124.00	166	35	21.08	2.02	5.51
Mean	1.41	0.24	1.35	0.74	0.30	0.13	39.33	45.00	6.00	191.00	93.33	149.00	49.00	32.89	2.01	5.6
GU026	1.45	0.29	1.44	0.90	0.31	0.16	66.00	40.00	6.00	210.00	112.00	183	135	73.77	2.13	5.49
GU045	1.21	0.28	1.40	1.29	0.44	0.17	46.00	39.00	6.00	151.00	128.00	326	152	46.63	2.12	6.52
GU607	1.34	0.27	1.63	1.16	0.36	0.13	53.00	43.00	8.00	203.00	252.00	265	158	59.62	1.81	5.32
Mean	1.33	0.28	1.49	1.12	0.37	0.15	55.00	40.67	6.67	188.00	164.00	258.00	148.33	57.49	2.02	5.77
TAG31	1.48	0.27	1.13	1.05	0.31	0.17	33.00	61.00	8.00	167.00	81.00	119	43	36.13	1.80	4.93
Mean	1.48	0.27	1.13	1.05	0.31	0.17	33.00	61.00	8.00	167.00	81.00	119.00	43.00	36.13	1.80	4.93
Trial Mean	**1.40**	**0.25**	**1.31**	**1.12**	**0.33**	**0.13**	**39.58**	**50.17**	**6.67**	**173.75**	**108.83**	**173.50**	**93.83**	**54.08**	**2.03**	**5.75**

The three separate data sets were combined for analysis purposes to reduce the amount of variability that would have arisen from having too few degrees of freedom (d.f. = 23). Analysis of variance (ANOVA-one way, no blocking), results are shown in Table 6.7.

Table 6.7 ANOVA of nutrient differences between clones

	N %	P %	K %	Ca %	Mg %	Na %	B mg/kg	Cu mg/kg	Fe mg/kg	Zn mg/kg	Mn mg/kg
NH000	1.89	0.307	1.420	0.530	0.283	0.133	52.70	10.00	117.70	56	268
GN107	2.09	0.313	1.407	0.473	0.223	0.137	68.30	10.33	90.70	67	273
GN156	2.31	0.350	1.647	0.550	0.253	0.097	76.70	10.33	106.00	80	296
GU026	2.42	0.343	1.737	0.623	0.313	0.153	93.00	11.33	110.00	88	256
GU045	1.86	0.350	1.867	0.887	0.41	0.150	70.30	9.67	152.70	77	177
GU607	2.38	0.340	1.863	0.777	0.337	0.140	74.70	11.67	224.30	76	234
GC627	2.03	0.250	1.500	0.973	0.303	0.073	59.70	9.67	108.30	118	174
TAG031	2.22	0.317	1.343	0.727	0.300	0.163	70.30	12.33	103.00	128	196
F- values	n.s.	n.s.	n.s.	n.s.	**	*	n.s.	n.s.	**	n.s.	n.s.

n.s. = not significant; * = 5%; ** = 1%

Elemental differences (magnesium and iron) were evident at the 1% level in clone GU045 and GU607 respectively. There were also clonal differences (clone GC627) for sodium at the 5% level. All other elements showed no significant variations at the clone level. A multiple linear regression model was determined for the relationship between rooting and nutrients, and nutrient ratios to identify those with the greatest interaction. Utilising all the elements as explanatory variables, the model could account for **42.1%** (d.f. = 23) of the variance with a standard error of **15.1**. Calcium (r = 0.507) and magnesium (r = 0.569) correlated moderately with rooting.

For the same data, a step-wise regression produced a very interesting output that accounted for **60.3%** of the variance, the highest ever achieved in these series of experiments, whilst the standard error dropped to **12.5**. The elements identified as forming the best model included calcium, potassium and zinc. The resultant equation for rooting was:
rooting % = - 56.0 + 55.92(Ca) + 26.7(K) + 0.1668(Zn). Overall the model can be regarded as fairly strong because of the higher value of variance accountability (60.3%), and once again calcium and zinc were identified as interacting with clonal eucalypt rooting.

6.3.5 Overall rooting responses

The mean rooting for the experiment was 39.85%. The high mean rooting of the first harvest (46.36%) was unexpected and did not follow the normal pattern of slowly improving with successive harvests. The major 'dip' in mean rooting (Table 6.8) at the second harvest (29.41%) is an oddity that was observed in the previous experiment (Chapter 5), where the second harvest rooting results were also lower than the first. However, the third harvest (Figure 6.4) showed a major improvement (mean = 54.08%), and followed the same improved rooting trend observed in the previous experiment at the third harvest (Chapter 5).

Table 6.8 Summary of mean rooting over three harvests

Summary Statistic	Harvest 1	Harvest 2	Harvest 3
Mean	46.36%	29.41%	54.08%

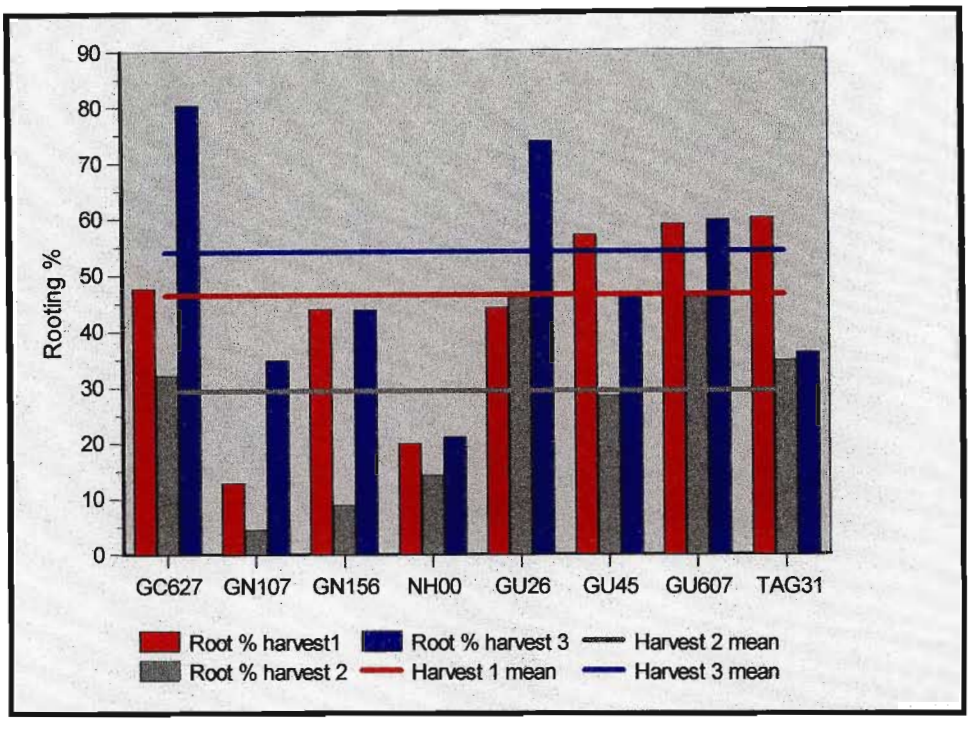
Figure 6.4 ***E. grandis x E. camaldulensis*** **rooted cuttings prior to destructive sampling at final harvest**

The hydroponic system (NFT) did not change the ranking order of the test clones and the findings were directly in line with those from our commercial clonal nurseries.

The order of ranking for the experiment was (mean rooting %) (Figure 6.5):

1. *E. grandis x E. camaldulensis* 53.40% (Figure 6. 4).
2. *E. grandis x E. urophylla* 51.22%
3. *E. grandis* 43.62%
4. *E. grandis x E. nitens* 22.71%.

Figure 6.5 Comparison of rooting of test clones at three separate harvest times



6.3.6 Change in nutrient concentration with time

Two sets of tissue analysis data were collected over a 121 day period, one set in September 2001 and the last in January 2002. To explain more of the variation in differences in nutrient concentration, a one-way ANOVA (in randomised blocks, d.f. = 15) was run. With data sets as block strata and clones as plots (main effects), it was possible to calculate and identify the variation of the plots within the blocks and the resultant interactions over the 121 days (Table 6.10).

Table 6.9 ANOVA for change in nutrient concentration with time

	N %	P %	N:P	N:K	K %	P:K	Ca %	Mg %	Na %	Ca:Mg	B mg/kg	Cu mg/kg	Zn mg/kg	Mn mg/kg
Clone	n.s	**	n.s	n.s	n.s	n.s	n.s	**	*	n.s	n.s.	n.s	n.s	n.s
S.e.d.	0.289	0.015	0.025	0.109	0.183	0.588	0.166	0.024	0.142	0.052	11.970	1.065	11.970	43.700
Direction of change	n.s	-	n.s	n.s	n.s	n.s	n.s.	+	-	n.s	n.s	n.s	n.s	n.s

n.s. = not significant; * = 5%; ** = 1%; s.e.d = standard error of difference.

The ANOVA test identified three changes in elemental concentration over the 121 day period (Table 6.9). Magnesium showed a significant difference (1% level) over the period. The variation was evident in clone GU45 and magnesium increased in concentration over time. Sodium was significant at the 5% level and the concentration change decreased with time. Clone GC627 accounted for the greatest variance. Clone GC627 also accounted for the

significant difference of phosphorus, and the concentration levels decreased during the 121 day interval.

6.4 CONCLUSION

There was a 1.59 fold increase in cuttings from the first to the second placement, whilst no noticeable increase occurred from the second to third harvest. The negligible change in cutting yield between the last two harvests may be due to the earlier onset of an increase in the root collar diameter of hedge ramets in response to the wider espacement between plants. Although not measured, a visual evaluation of the stems indicated a rapid thickening of stems from the first to the second harvest, and practical experience has shown that the greater the RCD, the higher the yield of coppice material. Correlation values for cutting length and root collar diameter (RCD) revealed that differences were due to clone type. Rooting correlated weakly with both cutting length and RCD and neither variable can be used as predictive tools for rooting potential.

Rooting differences as a response to genotype were significant over the three harvests. This was an expected outcome that corresponded to the results from conventional field hedges and previous hydroponic experiments. The hydroponic system (NFT) did not affect the ranking of test clones (based on rooting %) and the order remained (1) *E. grandis x E. camaldulensis* (2) *E. grandis x E. urophylla* (3) *E. grandis* (4) *E. grandis x E. nitens.*

A step-wise regression identified calcium, potassium and zinc as interacting with rooting. Overall the model can be regarded as fairly strong due to the higher value of variance accountability, and once again calcium and zinc were identified as having a level of interaction with rooting. None of the element ratios interacted with rooting as was previously reported (Chapter 5). This may have been as a result of the variety of genotypes tested in this experiment, whilst Chapter 5 dealt only with the *E. grandis x E. nitens* cross. Specific models will have to be developed for each eucalypt hybrid cross if any precision in rooting predictability is to be achieved and the model equation from this experiment can be viewed as a 'generalist' indicator only.

The grand mean rooting for the experiment of 39.9% was acceptable, taking into account the range of eucalypt genotypes tested. The higher mean rooting of the first harvest (46.36%) was unusual and did not follow the normal pattern of rooting improving with successive harvests until a 'rooting plateau' is reached. The drop in rooting performance in the second

harvest is a pattern that emerged in the previous experiment (Chapter 5). The reason for this unexpected trend is difficult to explain but could be linked to some form of 'physiological harvest shock' from the first coppice collection and a disruption in the production of those plant growth regulants involved in root initiation. There may be a 'time-lag' recovery that only reaches a form of regulation or stasis with regular harvest intervals.

The concentration of nutrients did not differ significantly from the first to the third harvests and is unlikely to be the reason for depressed rooting results. A more reasonable explanation could be the slow adaptation of the hydroponic ramet to its confined growing conditions and a progressive 'hardening' to continual coppice harvests. No data from the experiments can directly support this view and further work is needed to identify the reasons for the trend. A comparison of nutrient concentration changes over a 121 day period identified only magnesium, sodium and phosphorus as varying significantly. Strangely, none of these elements were identified in the step-wise regression model as interacting with rooting, and only magnesium showed any moderate correlation to rooting in the matrix. It is speculated that these three elements (Mg, Na and P) may have been acting as a 'blocking' mechanism to rooting and that only a concentration change in magnesium, sodium and phosphorus could allow the 'rooting co-factors' to initiate rooting.

There are a number of factors, both individual and interactive, that affect the ability of eucalypts to root at sustainable levels. We can be reasonably sure that amongst the eucalypt clones there is a strong genotypic effect on rooting. Certain hybrid crosses such as the sub-tropical *E. grandis x E. camaldulensis and E. grandis x E. urophylla* always root consistently higher than their more temperate counterpart, *E. grandis x E. nitens.* The next major improvement in rooting will only come about when those clones with the best rooting ability are introduced into commercial propagation systems. Hydroponics is really nothing more than a balanced 'platform' that allows for intensive clonal hedge management. According to TIBBIT *et al.* (1997), programmes of genetic improvement of *E. nitens* often have emphasis on breeding for growth and wood quality traits, with the possibility of vegetatively propagating superior trees for planting stock. Consequently, there has been some recent focus on understanding the mechanisms of clonal propagation of the species. Much of the research has focused on physiological and environmental factors. Indeed, these are paramount to the successful development of efficient procedures for raising cuttings. However, **genetic effects** also need to be understood in the whole strategy of clonally propagating a species which has been bred to produce elite genotypes.

CHAPTER 7

CONCLUSION

The vegetative propagation of eucalypt cuttings is a complex process that requires a sensitive balance of a number of physiological, genetic, edaphic, seasonal and environmental factors. FETT-NETO *et al.* (2001) describe rooting as an essential step in the vegetative propagation of economically important woody species that is affected by multiple factors including genetic characteristics, phytohormones, phenolic compounds and nutritional status, as well as associated stress responses such as wounding, changes in plant water relations and loss of correlative whole plant influences.

DE ASSIS (2001) states that the most important advantage of hydroponic mini-hedges is the potential to provide well balanced nutrition to hedge plants. Nutrition is a key factor affecting rooting predisposition, because of its involvement in determining the morphogenetic response of the plants. In hydroponic mini-hedges the nutrients can be supplied at ideal, customised, concentrations to promote this rooting. Hydroponics, without the influence of precipitation and overhead irrigation, allows better nutrition control of mother plants (stock plants). The use of covered hydroponic systems allows nutrient supply at correct concentrations and avoids the imbalances often experienced in field hedges.

COOPER (1996) has grown a number of different tree species using the Nutrient Film Technique (NFT) and found the stock plants to be of excellent vigour. He considers it to be an ideal system for the rooting of cuttings as the micro-environment can be carefully controlled and the efficiency of propagation enhanced. COOPER (1996) suggests that the aerial environment can be fully controlled by manipulating light, day and night temperatures, CO_2 content of the air and relative humidity. The use of the NFT for propagation purposes can provide a facility for precise control of the root-zone environment. The nutrient concentration, pH and growth regulator content of the solution at the cut surface can also be precisely controlled. With this degree of manipulation over both the aerial and root-zone environment, all that is theoretically necessary is to develop the optimum protocols to suit the specific crop.

The primary objective of the first experiment was to compare two hydroponic system designs. Although rooting was not significantly different, the trough system was cheaper to

manufacture, easier to manage and more space-efficient, accommodating up to 2.3 times more plants than the pot system. The experiment highlighted the need to reduce nutrient cycle times that induced stress through a constant saturation of the roots and the subsequent onset of *Botrytis sp.*

7.1 Environmental factors and their effect on rooting

Major environmental fluctuations must be removed if the hedge plants are to perform optimally and no matter where production takes place, it is imperative to control temperature extremes. Temperature can influence rooting by interfering with nutrient uptake and metabolism and its control must be adjusted for optimum cutting production. Considering that nutrient concentration is of prime importance in the rooting process, and that nutrient uptake depends on the metabolic activity, it must be assumed that both temperature and light contribute to the re-establishment of normal rooting competence (DE ASSIS, 2001).

Although not measured, the root growth of eucalypt hydroponic stock plants will be affected by cold weather and the absence of the moderating effect of soil on temperature could have deleterious effects on nutrient uptake. Studies of pine and spruce seedlings in hydroponic culture by VAPAAVUORI *et al.* (1992) have shown root growth to be inhibited, CO_2 uptake reduced, and changes in nitrogen translocation, at low root temperatures (5-8 ^{0}C).

Future decisions on the siting of a commercial hydroponic unit for the vegetative propagation of eucalypts will have to take greater cognisance of the effects of minimum winter temperatures than the effects of summer maximum temperatures. Future designs could probably be of the intermittent flow type configuration, housed in a controlled tunnel environment. The costs of cooling a tunnel through the use of evaporative cooling pads, extractor fans and shade net are far lower than that of heating a structure of a similar size. The process of a nutrient solution circulating through a bed will also have a major beneficial effect on cooling of the plant root structure and this can be further augmented through the installation of an overhead misting system.

The effect of lighting on clonal hydroponic production requires a lot more attention. Whereas in the Northern Hemisphere, the problem of insufficient light during winter can be improved through supplemental lighting, the comparative South African conditions may receive too high a daily level of insolation that negatively impacts on rooting, but not necessarily on vegetative growth. The poor initial rooting results from the third trial to test the response of three *E.*

grandis x E. nitens hybrid crosses to different nutrient solutions were similar to those experienced from first harvests in conventional clonal field hedges. However, the results were somewhat perplexing when compared to the good initial rooting results achieved in the first two experiments carried out under artificial lighting. Whereas it was initially thought that light intensity levels of 3000 to 4000 Lux were insufficient to sustain a hydroponic ramet, it now appears that it is quite sufficient for rooting purposes.

A slightly etiolated hedge plant may accumulate higher concentrations of carbohydrates in the stem, which could benefit rooting. This assumption must be tested by screening hydroponic beds with varying shade cloth densities. It may prove more practical to screen the hydroponic hedge plants with a dense shadecloth (75%) a week prior to the harvesting of coppice to induce etiolation. HANSEN (1987) believes that photoperiod combined with temperature can influence the rooting predisposition of shoots. Lighting can also influence rooting by controlling internal levels of carbohydrates. Etiolation of mother plants can affect rooting and root number.

This study would have been better able to account for the effect of light on hydroponically sustained eucalypt hedge plants if photo-active radiation (PAR) had been measured, and this must still be done. HOAD and LEAKEY (1992) found that in *E. grandis*, the higher rooting percentages recorded under lower R:FR (Red : Far Red) ratios, were associated with increased cutting lengths, higher specific leaf areas and higher rates of photosynthesis per unit leaf area during propagation. This relationship may be attributable to the capacity of the stem to store carbohydrates produced both pre- and post-severance.

7.2 Cutting dimensions and their effect on rooting

Rooting correlated weakly with cutting length and root collar diameter and both variables cannot be used to predict the rooting potential of hybrid eucalypts. The inclusion of the sturdiness ratio and the biomass index (both derived values) in the correlation of cutting dimensions with rooting response offered no further explanation for the lack of a linear relationship and were of no practical value.

7.3 Role of nutrient concentrations

The important role of foliar nutrient concentrations in eucalypts has been identified by a number of authors (CROMER *et al.*, 1981, SCHÖNAU,1981, SCHÖNAU and HERBERT, 1983). They can play an important role in the identification of nutritional deficiencies and

imbalances and are a powerful means of maximising productivity through nutritional amendment. There has now been a greater emphasis on identifying ideal ratios of nutrients in eucalypts.

In the last two experiments conducted, the nutrient ratios described by HERBERT (1996) were included to determine whether they interacted with rooting responses. Within the hybrid cross of *E. grandis x E. nitens*, certain element ratios were identified as being important (nitrogen : phosphorus, nitrogen : potassium and phosphorus : potassium), whilst over a range of eucalypt genotypes, they played no role at all.

7.4 Effect of genotype, substrate and system on nutrient concentrations

MULLIGAN (1988) notes that differences between nutrients in their distribution are related to differences in their physiological function and in their relative mobility within the plant. The distribution of nutrients between components of the plant also varies markedly between species, with the age or size and as a result of changes in the external nutrient supply.

Clone type, substrate and hydroponic system did not result in significant differences in the concentration levels of the macro-elements. However, there was significant variation in the concentration of three micro-elements (boron, manganese & zinc) as a result of system differences. The reason for the strong effect of the different systems on these micro-element concentrations cannot be accounted for.

7.5 Effect of genotype, substrate and fertiliser on rooting

AIMERS-HALLIDAY *et al.* (1999) note that in their clonal propagation of *E. grandis x E. nitens* in New Zealand, more cuttings were obtained from the hybrid *(E. grandis x E. nitens)* and *E. grandis* than from *E. nitens* stock plants. This indicated to them that the hybrid behaved more like *E. grandis* in ease of coppicing. The most important factor in determining the number of cuttings collected was the nutritional state. Stock plants that had fertiliser applied at the time of topping produced more cuttings than starved stock plants because the coppice shoots grew faster.

Although the introduction of hydroponic hedges for the production of high quality coppice material is a major technological development, it remains a system that has to be intensively managed and will not solve all the vagaries surrounding the propagation of difficult-to-root eucalypt hybrid crosses. Expectations from hydroponics in forestry propagation must be

tempered with the understanding that the benefits of the techniques are limited to managing those cultural factors affecting growth that were not possible in field hedges. However, hydroponics will not transform a poor-rooting eucalypt clone into some 'super-performer' overnight. It will allow a nurseryman to fully exploit and manipulate those cultural factors to obtain the very best rooting potential of the genotype. Rooting is a heritable factor and we need to acknowledge that some hybrid clones root well and others do not. Unless we are able to manipulate those genetic qualities determining the rooting propensity, there is very little that we can do to change that potential.

The substrates tested (perlite, sand and gravel) were not responsible for differences in cutting yield and rooting. Generally, rooting correlated weakly with fertiliser type and differences were not significant. However, as hedge ramets matured, their rooting response to fertiliser type started to fluctuate and indicated that nutrient uptake was possibly changing as plants physiologically aged or became accustomed to their specific nutrient feed. It is possible that further harvests would have clearly identified the best nutrient feed, but this was not evident after three consecutive harvests. From the experimental results, the recommended combination for the propagation of *E. grandis x E. nitens* is Hydroponica® fertiliser and gravel substrate. However, any well blended, balanced nutrient feed should suffice. Hoaglands No.2 and Chemicult® plant feed are both excellent alternatives and will provide similar rooting results. The deciding factor must be the availability of the nutrient blend and the costs. Unfortunately, Hoaglands has to be specifically formulated and is by far more expensive than Hydroponica®, Chemicult® or Natgro® that can be purchased straight from a supplier.

Four step-wise linear regression models highlighted those elements most strongly interacting with rooting and model variance accountability ranged from 36.9% to 60.3%. Unfortunately each experiment produced a different equation to explain nutrient interactions with rooting. However, in three out of four experiments, calcium and zinc were identified as playing a role in rooting response and more attention needs to be given to their role and function in root initiation in clonal eucalypts (Table 7.1). At this stage, one cannot clearly indicate which elements are the most predictive of rooting as it is most probable that the relationship is more likely to be a series of complex interacting elemental ratios.

Table 7.1 Commonality of nutrient element interactions and rooting response over four experiments (based on results from step-wise regression equations)

	N	N:P	N:K	P	P:K	K	Ca	Mg	Mn	Na	Cu	Zn	% variance
Exp. 1							X	X					36.9
Exp. 2											X	X	39.0
Exp. 3	X	X	X	X	X		X		X	X		X	51.7
Exp. 4						X	X					X	60.3

Regression calculations revealed that the different elements appeared to be changing concentration direction relative to one another and this 'push-pull' effect may be the catalyst that stimulates rooting. The variation in regression model outputs may also indicate that specific rooting models will have to be developed for each eucalypt hybrid cross if any degree of precision in rooting prediction is to be achieved.

It is possible that the optimum nutrient requirements for the production of wood fibre in a plantation and those required for the production of premium coppice (juvenile state) in a hydroponic hedge plant, may vary in terms of the different element concentrations.

7.6 Effect of fungal diseases

Fungal attack by plant pathogens poses a constant threat to the success and survival of an intensive clonal hydroponic system. A major loss of hydroponic hedge plants was caused by *Botrytis* sp. in the first experiment, as a result of too frequent a cycle of fertigation and it was discovered that the unequal cyclic timers should run for a maximum of 15 minutes 'on-time', for no more than four to five cycles per day. At higher application rates, roots started to turn brown and die back as a result of insufficient drainage. Powdery mildew (*Oidium eucalypti*) was a continual problem that plagued all four experiments and was never successfully controlled with a chemical spraying programme. A weekly spraying programme of fungicides is essential and alternate spray programmes using both contact and systemic types must be incorporated.

7.7 General recommendations.

The management of hydroponic mini-hedges is rather simple, but some aspects must be considered for the technique to be successful. The harvest of cuttings must be done selectively to avoid hedge plant degradation. Desalination (leaching) should be done at least once every 30 days by generously irrigating the substrate to leach out surface-accumulated salts. In non-automated systems, the entire solution should be changed every 15 days. DE ASSIS (2001) have found that with an intermittent flooding system (ebb and flow) better

results were obtained when the electrical conductivity (EC) was adjusted to between 1.8 and 2.2 mS/cm in winter and 0.8 to 1.0 mS/cm in summer (DE ASSIS, 2001). The experience in Mondi supports that of the Brazilians and fertigating at levels of over 1.3 mS/cm during summer causes hedge plants to develop very leafy growth with weak stems and long internodes. Reducing the EC level to between 0.7 and 0.8 mS/cm produces much better quality coppice. The number of nutrient cycles per day must be carefully monitored and linked to the prevailing weather conditions. On hot summer days where temperatures under the plastic tunnel can rise to 37 ^{0}C, three to four feeding cycles would be appropriate. Each cycle should run for at least 25 to 30 minutes. During winter, the number of cycles can be reduced to one and this must be completed before two o'clock in the afternoon to allow the substrate to drain before evening cold sets in.

It is important that hygiene receives a special focus and no abscised and cut leaves must be allowed to fall into the nutrient solution as this can spread pathogens. A chemical spray programme is essential and a rotational spray of contact and systemic fungicides needs to be applied at least once a week.

7.8 Future prospects

Although the production expectations from a hydroponic clonal garden must not be exaggerated, there is no doubt that the future of clonal propagation of eucalypts lies with intensively managed hydroponic mini-hedges grown within a highly controlled tunnel environment. The numerous benefits of recirculating hydroponics far outweigh the initial capital outlay and there is a major shift in South American forestry companies from conventional clonal field hedges to hydroponic mini-hedges. South African forestry will follow this trend and Mondi Forests has completed a commercial system at Mountain Home Nursery, and has already started construction on a large scale commercial ebb and flow system at Kwambonambi. This will eventually replace the extensive field hedges. More research needs to be done into hydroponic systems specifically suited to clonal forest nurseries and not necessarily relying on hybridised horticultural type units. Recirculating hydroponic technology will definitely lead the way due to its efficient utilisation of water and nutrients and the fact that it is environmentally friendly. Ebb and flow or intermittent flow technology seems to be the most efficient route to follow, but there is great potential in aeroponic systems, and research into its applicability to clonal eucalypt propagation is already well advanced on two Continents. The obvious focus must be on ways to reduce the

high capital outlay on equipment that could stifle progress in this direction for the smaller private companies.

Although this study has highlighted that rooting response to different nutrient feeds was not significant, it was by no means exhaustive or comprehensive enough. Further rooting gains could be achieved through the development of eucalypt-specific nutrient formulations. There have been major developments in the use of humates in hydroponic tomato production. VASILENKO (2002) describes humates as the salts of humic acids derived from the remains of plants and animals from millions of years ago. Among the various sub-fractions of the humates, the division between humic acid and fulvic acid fractions is of most importance. Fulvic acids are generally more plant active because of their higher oxygen content, and because of the abundance of carboxyl (COOH) groups. Humates are known to improve vegetative growth, increase the respiration rate of plants and increase the chlorophyll density of the leaves creating a higher photosynthetic rate. They also improve the overall nutrient uptake of the plant and theoretically, less nutrient feed would be required for greater growth and possibly rooting gains.

Hydroponics must offer the opportunity to scientists to better understand the role that plant growth regulators play in root initiation and it may be possible to significantly improve rooting results by the addition of IAA-type formulations to the recirculating nutrient solution. The benefits of vitamins, bio-stimulants and beneficial plant organisms such as *Trichoderma* must also be explored utilising this technology.

To fully exploit the potential gains that hydroponics offers to clonal forestry, a lot more attention will have to be given to the source and quality of the stock plants. Ramets derived from macro-cuttings have proven to be inferior to their tissue-culture-derived equivalents in terms of root development and stem straightness. Tissue culture plants phenotypically resemble a seedling and share similar fibrous root systems. Their growth response to feeding in a hydroponic bath is superior to the macro-derived plant and their recovery from coppice harvest is also better. To fully exploit the beneficial effects of juvenile stem cuttings on rooting, a lot more research will be needed to commercially introduce temperate eucalypt hybrids via the *in vitro* route.

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APPENDIX A

A BRIEF HISTORY OF HYDROPONICS AND THE CONCEPT OF ELEMENT 'ESSENTIALITY' DISCOVERED

A.1 History of hydroponics

Perhaps the earliest recordings of hydroponics were the Hanging Gardens of Babylon, where plants were grown in a steady stream of water. The floating gardens of the Aztecs and those of the early Chinese are further examples of hydroponics culture. Egyptian hieroglyphic records dating back several hundred years BC describe the growing of plants in water. Before the time of Aristotle, Theophrastus (372 - 328 BC) undertook various experiments in crop nutrition and botanical studies by Dioscorides date back to the first century AD (RESH,1998).

A.2 The discovery of plant nutrition

Since the Belgian Jan Van Helmont, in 1600, showed that plants obtain 'substances' from water, numerous researchers have busied themselves with gaining a better understanding of plant nutrient uptake. Although Van Helmont correctly surmised that plants obtain growth substances from water, he failed to identify that carbon dioxide (CO_2) and oxygen (O_2) were also essential. In 1699 John Woodward, an Englishman, grew plants in water containing various types of soil. He concluded that plant growth was as a result of certain substances in the water, derived from the soil, rather than solely the effects of water itself (RESH,1998). In 1804, De Saussure propounded that plants are composed of chemical elements obtained from water, soil and air, and in 1851, a French chemist, Boussingault, verified this through his experiments with plants grown in quartz, sand and charcoal, to which were added solutions of known chemical composition. Boussingault concluded that water was essential in providing hydrogen (H_2) and that plant dry matter consisted of hydrogen, carbon and oxygen. He also stated that plants contain nitrogen and other elements (RESH,1998).

The elimination of the substrate entirely and the growth of plants in a water solution containing minerals, was accomplished by two German scientists, Sachs in 1860 and Knop in 1861 (RESH,1998). The two German physiologists made synthetic solutions of essential plant nutrients in water. Included in these solutions were the essential elements nitrogen, phosphorus, sulphur, calcium, magnesium, potassium and iron. At the time, not enough was

known about trace elements and they were omitted. The formulae developed by these men are still applicable today (HARRIS, 1987).

A.3 The concept of 'essentiality' proposed

In 1939, Arnon and Stout, two plant physiologists at the University of California, identified three criteria for plant nutrient essentiality :

- Omission of the element in question must result in abnormal growth, failure to complete the life cycle, or premature mortality of the plant.
- The element must be specific and not replaceable by another.
- The element must exert its effect directly on growth or metabolism and not some indirect effect such as by antagonising another element present at a toxic level (BENTON-JONES, 1998).

Between 1922 and 1954, additional elements **essential** to plant growth were identified. These included manganese, copper, zinc, molybdenum, boron and chlorine. Although 40 years have passed since the last essential element, chlorine, was identified, plant physiologists are still actively engaged in determining what additional elements can be added to the list of 16 (Table A.1). In 1983, Eskew, Welsh and Cary suggested that Nickel should be included as an essential element. (BENTON JONES, 1998).

Table A.1 Discovery of elements and their 'essentiality'

Element	Discoverer	Year	Discoverer of essentiality	Year
C	Known since ancient times	?	DeSaussure	1804
H	Cavendish	1766	DeSaussure	1804
O	Priestley	1774	DeSaussure	1804
N	Rutherford	1772	DeSaussure	1804
P	Brand	1772	Ville	1860
S	Known since ancient times	?	von Sachs, Knop	1865
K	Davy	1807	von Sachs, Knop	1860
Ca	Davy	1807	von Sachs, Knop	1860
Mg	Davy	1808	von Sachs, Knop	1860
Fe	Known since ancient times	?	von Sachs, Knop	1860
Mn	Scheele	1774	McHargue	1922
Cu	Known since ancient times	?	Sommer, Lipman & MacKinnon	1931
Zn	Known since ancient times	?	Sommer & Lipman	1926
Mo	Hzelm	1782	Arnon & Stout	1939
B	Gay Lussac & Thenard	1808	Sommer & Lipman	1926
Cl	Scheele	1774	Stout	1954

(BENTON-JONES, 1998)

A.4 The commercial development of hydroponics

Between 1925 and 1935, extensive progress took place in modifying the laboratory techniques of early form of hydroponics ('nutriculture') and applying it to large scale crop production (RESH,1998).

In the early 1930's, WF Gericke of the University of California put laboratory experiments in plant nutrition on a commercial scale. He coined the term *hydroponics*. The word was derived from the Greek words *hydro* (water) and *ponos* (labour) (RESH,1998). By means of water culture, Gericke grew tomato plants to such heights that ladders were necessary to pick the fruit, whilst the yields were quite spectacular (HARRIS, 1987). Unfortunately, the American press made widely irrational claims, calling it the discovery of the century. This lead to a number of unscrupulous people trying to cash in on the concept by selling useless equipment (RESH, 1998).

During the second world war, the United States Air Force stationed in the Pacific Islands had the problem of providing its personnel with fresh produce. The first commercial scale hydroponics systems was developed in 1945 on Wake Island. The worlds largest hydroponics installation was the US Army's 22 ha project on Chofu Island, Japan. On this installation was the world's largest greenhouse, covering 21370 m^2, containing 87 beds, each 91 m long by 1.25 m wide. The growing medium used was gravel (HARRIS, 1987).

Large hydroponics installations are to be found in Italy, Spain, France, England, Germany and Sweden. Hydroponic vegetable growing is also common in the arid areas of the Arabian Gulf and Israel (HARRIS, 1987).

APPENDIX B

SOUTH AFRICAN FORESTRY IN THE SOUTHERN AFRICAN AND INTERNATIONAL CONTEXT

B.1 Historical development of South African forestry

OWEN and VAN DER ZEL (2000) state that in the Southern Hemisphere, South Africa has the third largest and one of the oldest plantation resource areas. South African forests gained real significance in 1652, with the arrival of the Dutch at the Cape and again in 1820 when the English arrived in the Eastern Cape and Natal. The woodlands increased in area with the colonisation of the interior after 1836. It was not until 1875 that the first real planting of exotic timber species began, spurred on by the need for fuel wood for the fledgling railways. *Acacia mearnsii (*Black wattle) was introduced from Australia in 1864 and by 1880 was recognised as a superior tanning material. By 1910 an area of 60 000 ha had been planted to wattle.

The first Forest Act, for the Cape Colony, was promulgated in 1888 after the appointment of the first formally trained forest officer in 1880. Prior to the Union in 1910, there were four provincial forest services, with the most active in the Cape Colony. By 1938, it was recorded that 150 000 ha of commercial timber plantations had been planted by the state, whilst private growers had established 370 000 ha, including 220 000 ha under wattle. Up until the second world war, forestry was almost exclusively a State affair and strongly linked to agriculture. The one exception was the wattle industry in KwaZulu-Natal, which was largely private.

After the second world war an independent Department of Forestry was established. It pursued a protectionist strategy towards the remaining closed canopy forests, while moving forward with the establishment of man-made timber plantations. The second world war emphasised South Africa's vulnerability in respect of timber supplies and in the early 1950's the State set about expanding the timber resources, especially by encouraging the private sector to plant trees. By 1960 the total planted area had reached 981 640 ha, of which 720 320 ha, including 355 000 ha wattle, was privately owned. By 1975 the total planted area had reached just over 1.1 million ha, of which 769 000 ha was privately owned. The area under wattle had shrunk to 161 000 ha. (OWEN and VAN DER ZEL, 2000).

OWEN and VAN DER ZEL (2000) state that by 1985, the Department of Forestry controlled 1.6 million hectares of land, of which 263 000 ha was under commercial plantations. In terms of the Government policy of the day, the eight independent homelands controlled a further 350 000 ha of land of which 150 000 ha was under commercial plantations. The official public planted area was 333 776 ha whilst the area under private ownership was 800 000 ha. In 1986, one million hectares of unplanted state forest mountain catchment area was transferred from the Department of Forestry to the provincial nature conservation authorities.

In 1990 the government commercialised its timber production activities. The South African Forestry Company Limited (SAFCOL) took over approximately 500 000 ha of state forest land, of which 263 000 ha was planted to timber plantations. The official total planted public area was 418 023 ha. The plantation area under private ownership was 952 870 ha, including a wattle area of 124 117 ha. The most recent statistics (DWAF August 1998) in respect of planted timber plantations and processing plants are reflected below:

Total plantation area	Public land	Private - Company	Private - Farmers	Softwoods	*E. grandis*	Other eucalypts	Wattle	Other
1 518 138 ha	455 541 ha (30%)	743 270 ha (49%)	319 327 ha (21%)	797 610 ha 52.6%	441394 ha 29%	156 570 ha 10.3%	112 029 ha 7.4%	10 535 ha 0.7%

(OWEN and VAN DER ZEL, 2000)

B.2 South African forestry in the Southern African and international context.

In the Southern Hemisphere, South Africa has the third largest and one of the oldest plantation resource areas. Half of Africa's plantation areas are in South Africa and it is widely regarded as among the world leaders in the management of, and research on, man-made timber plantations.

A South African total plantation area of 1.5 million ha, consisting of equal areas of softwood and hardwood species of comparative yields, produces both softwood saw timber (from the world's largest pruned softwood resource) and strategically developed softwood and hardwood pulpwood and pulp products (OWEN and VAN DER ZEL, 2000).

Two large pulp companies, Mondi Forests and Sappi Forests, own large timber estates and processing plants in Swaziland and manage these on an integrated basis. Imports of softwoods from Zimbabwe have increased steadily to 60 000 m^3 per year and could increase to at least 100 000 m^3 per year in the near future, because of the closure and rationalisation of South African sawmills.

Other countries in Southern Africa have a combined plantation area of nearly 500 000 ha, but quality and growth rates of these do not compare favourably with South Africa, resulting in a slower processing development.

An important feature of South African forestry, by comparison with both SADC and the rest of the world, is that both in the fields of timber growing and processing, the private sector plays a pivotal and leading role. Seventy (70) percent of the plantation area and 90% of the processing capacity is managed by private growers and processors. The plantation forestry sector is an important primary employer of people in rural areas, while the forests products is the fourth largest manufacturing sector in the country.

Although South Africa has only 0.07% of the world's productive forest areas, it still produces 1.2% of the global industrial output. The plantations produced (1996/97) 18.6 million m^3 of logs, at a value of R1.75 billion, while the primary processing industry earned R9.15 billion through the sales of sawn timber, pulpwood, mining timber, panel products, poles, charcoal, chips and firewood (OWEN and VAN DER ZEL, 2000).

APPENDIX C

COMPARISON OF EXPERIMENTAL RECIRCULATING HYDROPONIC SYSTEMS

C.1 Trial randomisation

C.1.1 Pot system

1.(plot) Sand (substrate) NH000 (Clone ID) 7cm (Planting Height)	18. Gravel GN107 7cm	19. Perlite GN156 5cm	36. Perlite GN107 5.5cm
2. Gravel GN156 5cm	17. Gravel GN107 6cm	20. Gravel GN107 5cm	35. Perlite NH000 5cm
3. Perlite GN107 6cm	16. Perlite NH000 4cm	21. Gravel GN156 6cm	34. Sand NH000 3.5cm
4. Sand GN107 5.5cm	15. Sand GN107 5.5cm	22. Sand GN107 5cm	33. Gravel GN107 5cm
5. Perlite GN156 8.5cm	14. Sand NH000 6.5cm	23. Gravel NH000 4cm	32. Sand GN156 5.5cm
6. Gravel NH000 6.5cm	13. Sand NH000 5.5cm	24. Perlite GN156 7.5cm	31. Gravel GN156 5cm
7. Gravel GN156 6cm	12. Gravel NH000 6cm	25. Sand GN107 5cm	30. Perlite GN107 3.5cm
8. Perlite NH000 5cm	11. Perlite GN107 5.5cm	26. Gravel NH000 6cm	29. Sand GN156 5cm
9. Perlite GN156 6cm	10. Sand GN156 7.5cm	27. Perlite NH000 4cm	28. Sand GN156 6cm

C.1.2 Trough system randomisation

GRAVEL	SAND	PERLITE	NFT
1 GN107 4cm	16 NH000 7cm	31 GN156 7cm	46 NH000 6cm
2 GN156 7cm	17 GN156 6cm	32 NH000 4.5cm	47 GN156 6.5cm
3 NH000 6cm	18 GN107 7cm	33 GN156 5cm	48 GN156 4.5cm
4 NH000 6cm	19 GN107 5.5cm	34 GN156 6cm	49 GN156 6.5cm
5 NH000 4.5cm	20 GN156 7.5cm	35 NH000 4.5cm	50 NH000 6cm
6 GN156 4.5cm	21 GN107 6.5cm	36 GN156 4.5cm	51 GN107 3cm
7 GN107 5cm	22 NH000 4.5cm	37 GN107 5cm	52 GN107 6cm
8 GN107 5cm	23 NH000 5cm	38 NH000 6cm	53 GN156 6cm
9 NH000 5cm	24 GN107 5.5cm	39 GN107 5cm	54 NH000 5.5cm
10 NH000 5cm	25 GN156 5.5cm	40 NH000 5.5cm	55 GN107 6.5cm
11 GN107 6cm	26 GN107 4.5cm	41 NH000 7cm	56 GN107 6.5cm
12 GN156 5.5cm	27 NH000 7cm	42 GN156 5cm	57 NH000 5cm
13 GN156 4.5cm	28 GN156 4cm	43 GN107 6.5cm	58 GN156 5.5cm
14 GN107 6cm	29 GN156 5.5cm	44 GN107 6cm	59 GN107 5cm
15 GN156 4cm	30 NH000 5cm	45 GN107 5cm	60 GN107 7cm
-	-	-	61 NH000 5cm
-	-	-	62 GN156 5cm
-	-	-	63 GN107 4.5cm
-	-	-	64 GN156 6cm
-	-	-	65 NH000 6cm
-	-	-	66 NH000 5cm

C.2. Temperature measurements

C.2.1 Minimum and maximum ambient temperatures

Date	Time	Pot min. temp.(°C)	Pot max. temp.(°C)	Gutter min. temp.(°C)	Gutter max. temp.(°C)	Air temp.(°C)
24/6/99	9:45	11	18	11	18	16
24/6/99	13:00	14	23	14	23	23
24/6/99	15:30	17	22	17	22	17
25/6/99	9:30	15	13	15	13	15
25/6/99	13:10	14	15	14	15	15
25/6/99	15:20	14	15	14	15	14
28/6/99	8:35	8	18	8	18	10
28/6/99	13:20	10	20	10	20	20
28/6/99	15:25	20	20	20	20	20
29/6/99	8:20	9	19	9	19	12
29/6/99	13:20	11	21	11	21	21
29/6/99	15:55	20	20	20	20	20
30/6/99	8:20	12	19	12	19	14
30/6/99	14:00	14	18	14	18	18
30/6/99	15:45	17	17	17	17	17
1/7/99	8:05	10	15	12	15	12
1/7/99	16:20	12	19	12	20	17
2/7/99	8:00	9	18	9	18	10
2/7/99	16:25	10	15	11	18	14
5/7/99	8:00	9	19	9	23	10
5/7/99	15:55	11	19	12	19	18
6/7/99	8:00	9	18	12	18	12
6/7/99	16:00	11	20	12	20	20
7/799	8:00	9	19	10	19	10
7/7/99	16:10	10	19	12	20	19
8/7/99	8:10	9	19	10	19	12
8/7/99	15:30	11	20	12	20	20
9/7/99	8:05	9	19	10	19	12

C.2.2 Substrate and ambient temperatures (pot system)

Day	Time	Air temp (°C)	Min. temp (°C)	Max. temp (°C)	Pot temp - dry S (°C)	Pot temp - dry P (°C)	Pot temp - dry G (°C)	Pot temp - wet S (°C)	Pot temp - wet P (°C)	Pot temp - wet G (°C)
24/6	9:45	16	11	18	13	12	12	15	14	14
24/6	13:00	23	14	23	18	15	17.5	17	15	17
24/6	15:30	17	17	22	18	17	17	16	15	16
25/6	9:30	15	15	13	11	13	12	12	14	13
25/6	13:10	15	14	15	15	13	15	14	12	15
25/6	15:20	14	14	15	15	16	15	14	15	15
28/6	8:35	10	8	18	7	7	7	10	9	9
28/6	13:20	20	10	20	11	15	18	14.5	14.5	16
28/6	15:25	20	20	20	15	15	18	14	15	15
29/6	8:20	12	9	19	8	8	10	9	10	11
29/6	13:20	21	11	21	16	15	19	14	14	18
29/6	15:55	20	20	20	16	14	19	16	14	17
30/6	8:20	14	12	19	12	12	11	13	12	11
30/6	14:00	18	14	18	16	14	13	16	14	13
30/6	15:45	17	17	17	16	14	13	16	14	12

C.2.3 Minimum and maximum temperatures (gutter system) Numbers in brackets indicate position of thermometer in gutter at time of reading, S - Sand, G - Gravel, P - Perlite

Day	Time	Air temp (°C)	Min. temp (°C)	Max. temp (°C)	Gutter temp-dry P (°C)	Gutter temp-dry S (°C)	Gutter temp-dry G (°C)	Gutter temp-wet P (°C)	Gutter temp-wet S (°C)	Gutter temp-wet G (°C)
28/6	8:15	10	8	18	(1) 8	(1) 8	(1) 9	(1) 10	(1)9.5	(1) 10
					(2) 6	(2) 7	(2) 7	(2) 7	(2)8.5	(2) 8
					(3) 7	(3) 8	(3) 7	(3) 8	(3) 8	(3) 8
28/6	13:20	20	10	20	(1) 17	(1) 12	(1) 18	(1) 15	(1) 16	(1) 15
					(2) 15	(2) 14	(2) 16	(2) 14	(2) 16	(2) 16
					(3) 16	(3) 16	(3) 18	(3) 15	(3) 15	(3) 16
28/6	15:15	20	20	20	(1) 17	(1) 17	(1) 18	(1) 15	(1) 16	(1) 15
					(2) 17	(2) 16	(2) 19	(2) 17	(2) 15	(2) 16
					(3) 15	(3) 16	(3) 17	(3) 15	(3) 16	(3) 16
29/6	8:10	12	9	19	(1) 9	(1) 10	(1) 10	(1) 11	(1) 10	(1) 11
					(2) 9	(2) 9	(2) 10	(2) 10	(2) 10	(2) 10
					(3) 8	(3) 10	(3) 10	(3) 10	(3) 8	(3) 10
29/6	12:50	21	12	21	(1) 14	(1) 16	(1) 16	(1) 13	(1) 14	(1) 14
					(2) 15	(2) 15	(2) 17	(2) 16	(2) 16	(2) 15
					(3) 15	(3) 16	(3) 16	(3) 16	(3) 17	(3) 17
29/6	15:45	20	20	23	(1) 18	(1) 16	(1) 18	(1) 14	(1) 14	(1) 15
					(2) 17	(2) 17	(2) 17	(2) 16	(2) 17	(2) 15
					(3) 18	(3) 18	(3) 19	(3) 17	(3) 17	(3) 18
30/6	8:10	14	12	19	(1) 11	(1) 13	(1) 12	(1) 12	(1) 13	(1) 12
					(2) 10	(2) 12	(2) 11	(2) 11	(2) 12	(2) 11
					(3) 12	(3) 13	(3) 12	(3) 12	(3) 11	(3) 12
30/6	13:50	18	14	18	(1) 14	(1) 14	(1) 15	(1) 15	(1) 16	(1) 15
					(2) 16	(2) 16	(2) 15	(2) 15	(2) 15	(2) 15
					(3) 14	(3) 15	(3) 16	(3) 15	(3) 14	(3) 15
30/6	15:35	17	17	18	(1) 14	(1) 15	(1) 16	(1) 14	(1) 13	(1) 15
					(2) 15	(2) 15	(2) 15	(2) 15	(2) 15	(2) 15
					(3) 16	(3) 15	(3) 16	(3) 15	(3) 16	(3) 16

C.3 Flow rates

C.3.1 Unadjusted flow rates pot system (23/6/99)

Pot no.	Flow rate (ml/sec)	Flow rate (ml/min)	Pot no.	Flow rate (ml/sec)	Flow rate (ml/min)
1	5.13	308	19	5	300
2	3.47	208	20	4.07	244
3	4.13	248	21	4.47	268
4	3.43	206	22	4.27	256
5	4.07	244	23	3.73	224
6	3.8	228	24	4.2	252
7	4.13	248	25	3.4	204
8	4.4	264	26	2.73	164
9	2.8	168	27	3.87	232
10	2.87	172	28	4.07	244
11	2.8	168	29	4.93	296
12	4.4	264	30	3.87	232
13	3.87	232	31	3.53	212
14	5.2	312	32	4.66	280
15	4.73	284	33	5.87	352
16	4.53	272	34	5.13	308
17	4.47	268	35	4.23	252
18	5.47	328	36	4.66	280

C.3.2 Adjusted flow rates pot system (23/6/99)

Pot no.	Flow rate (ml/sec)	Flow rate (ml/min)	Pot no.	Flow rate (ml/sec)	Flow rate (ml/min)
1	4.27	256	19	3.93	236
2	4.33	260	20	4.27	256
3	3.87	232	21	4.27	256
4	4	240	22	4.27	256
5	4	240	23	4.33	260
6	4.13	248	24	3.87	232
7	4.27	256	25	4.13	248
8	4.13	248	26	4.4	264
9	4.13	248	27	4.33	260
10	4.33	260	28	4.2	252
11	4.4	264	29	4.07	244
12	4.6	276	30	3.87	232
13	4.33	260	31	4.07	244
14	4.2	252	32	4.13	248
15	4.13	248	33	4.33	260
16	3.66	220	34	4.07	244
17	4.27	256	35	4.27	256
18	3.66	220	36	4.33	260

C.4 Temperature, EC and pH data

C.4.1 Temperature, EC and pH samples (pot & gutter, 28/6/99) unadjusted for pH

	Temperature (°C)	EC (µS/cm)	pH
Initial (Pot system)	12	80	9.41
Initial (Gutter system)	12	80	9.37
Perlite 1	12	80	9.14
Perlite 2	10	80	8.77
Perlite 3	11	80	9.1
Sand 1	11	70	8.3
Sand 2	12	70	8.2
Sand 3	12	70	8.48
Gravel 1	12	80	9.1
Gravel 2	11	80	9.07
Gravel 3	11	80	9.03
Discharge (Pot sys.)	12	80	9
Discharge (Gutter sys.)	11	80	8.75

C.4.2 Temperature, EC and pH samples (pot & gutter, 29/6/99) unadjusted for pH

	Temperature (°C)	EC (µS/cm)	pH
Initial (Pot system)	11	80	8.32
Initial (Gutter system)	12	80	8.76
Perlite 1	11	80	8
Perlite 2	11	80	8
Perlite 3	11	80	7.97
Sand 1	12	80	7.98
Sand 2	12	80	7.87
Sand 3	12	70	7.66
Gravel 1	12	80	8.03
Gravel 2	12	80	7.98
Gravel 3	12	80	7.95
Discharge (Pot sys.)	12	80	7.95
Discharge (Gutter sys.)	11	80	7.87

C.4.3 Temperature, EC and pH samples (pot & gutter, 30/6/99) unadjusted for pH

	Temperature (°C)	EC (µS/cm)	pH
Initial (Pot system)	13	80	8.18
Initial (Gutter system)	12	80	9.12
Perlite 1	13	80	8.44
Perlite 2	13	80	8.37
Perlite 3	13	80	8.4
Sand 1	12.5	80	8.3
Sand 2	12	80	8.44
Sand 3	13	80	8.45
Gravel 1	13	80	8.46
Gravel 2	12.5	80	8.47
Gravel 3	13	80	8.49
Discharge (Pot sys.)	13	80	8.41
Discharge (Gutter sys.)	13	80	8.97

C.4.4 Temperature, EC and pH samples (pot & gutter, 2/7/99) unadjusted for pH

	Temperature (°C)	pH	EC (µS/cm)
Initial (pots)	15	6.77	1,150
Initial (gutters)	14.9	6.72	1,220
Perlite 1	14.9	6.76	1,140
Perlite 2	15.2	6.78	1,150
Perlite 3	15.1	6.77	1,150
Sand 1	15.3	6.71	1,150
Sand 2	15.3	6.76	1,160
Sand 3	15.4	6.75	1,170
Gravel 1	15.3	6.76	1,150
Gravel 2	15.3	6.76	1,160
Gravel 3	15.5	6.76	1,160
Discharge (pots)	15	6.84	1,150
Discharge (gutters)	14.6	6.74	1,170
Static (pots)	15.2	6.78	1,100
Static (gutters)	14.4	6.74	1,220

C.5 Comparison of EC strength for varying concentrations of Hydroponica® + calcium nitrate (pot system, 30/6/99) unadjusted for pH

	EC_{35g} (µS/cm)	EC_{36g} (µS/cm)	EC_{46g} (µS/cm)	EC_{50g} (µS/cm)	pH_{50g}
Time	9:00	11:00	13:00	15:00	
Initial	1,100	920	1,100	1,210	6.8
Perlite 1	770	870	1,090	1,220	6.8
Perlite 2	730	890	1,040	1,190	6.79
Perlite 3	350	870	990	1,090	6.79
Sand 1	910	910	1,160	1,200	6.78
Sand 2	210	930	1,160	1,190	6.77
Sand 3	950	920	1,090	1,190	6.77
Gravel 1	1,030	910	1,180	1,200	6.78
Gravel 2	910	910	1,170	1,190	6.77
Gravel 3	960	910	1,160	1,200	6.77
Discharge	910	920	1,100	1,210	6.8
Static				1,130	6.88

(EC_{35g}: 35 g of Hydroponica® + 35 g of calcium nitrate mixed in 90 L water.)

C.6 Temperature, pH and EC readings for fertiliser concentrations to determine the volume of water (Hydroponica® and calcium nitrate) unadjusted for pH

	Temperature (°C)	pH	EC (µS/cm)
1	19.7	3.23	11.4 mS
2	21.1	7.34	11.5 mS
3	21.8	2.97	19.90 mS +
4	21.6	5.68	2.6 mS
5	19.8	6.34	1340 µS
6	18.7	6.65	910 µS
7	19.4	6.53	1090 µS
8	19.5	6.45	1260 µS

Temperature, pH and EC readings were taken for various concentrations to determine the ratio of water to Hydroponica® and calcium nitrate. Unadjusted pH.

1: 10 g Hydroponica® + 1 L water
2: 10 g calcium nitrate + 1 L water
3: 10 g Hydroponica® + 10 g calcium nitrate + 1 L water
4: 10 g Hydroponica® + 10 g calcium nitrate + 10 L water
5: 10 g Hydroponica® + 10 g calcium nitrate + 20 L water
6: 7 g Hydroponica® + 7g calcium nitrate + 20 L water
7: 8 g Hydroponica® + 8 g calcium nitrate + 20 L water
8: 9 g Hydroponica® + 9 g calcium nitrate + 20 L water
+ Value was greater than EC meter capacity.

C.7 Infiltration rates determined for media type

	Height (m)	Time (sec)	Infiltration (m/s)
Perlite 1	0.145	210	0.00069
Perlite 2	0.145	200	0.00073
Perlite 3	0.145	235	0.00062
Perlite average	0.145	215	0.00067
Sand 1	0.145	125	0.00116
Sand 2	0.145	90	0.0016
Sand 3	0.145	100	0.00145
Sand average	0.145	105	0.00138
Gravel 1	0.145	75	0.0019
Gravel 2	0.145	75	0.0019
Gravel 3	0.145	85	0.0017
Gravel average	0.145	78.33	0.00185

C.8 Rooting results for pot system - sorted by substrate

Plot No.	Treatment	Clone No.	Status	Cuttings placed	Cuttings rooted	% root strike
17	gravel	GN107		2	0	0
18	gravel	GN107		4	1	25
20	gravel	GN107		4	2	50
33	gravel	GN107		3	2	67
2	gravel	GN156		2	0	0
21	gravel	GN156		4	3	75
31	gravel	GN156		4	3	75
7	gravel	GN156		3	1	33
12	gravel	NH000		3	1	33
23	gravel	NH000	Dead - ***Pythium***	0	0	0
26	gravel	NH000	Dead-cutback mortality	0	0	0
6	gravel	NH000	Dead - ***Pythium***	0	0	0
11	perlite	GN107		2	2	100
3	perlite	GN107	Dead - ***Pythium***	0	0	0
30	perlite	GN107		4	3	75
36	perlite	GN107		6	6	100
19	perlite	GN156	Dead-cutback mortality	0	0	0
24	perlite	GN156		1	0	0
5	perlite	GN156	Dead - ***Pythium***	0	0	0
9	perlite	GN156	Dead-cutback mortality	0	0	0
16	perlite	NH000		4	3	75
27	perlite	NH000	Dead-cutback mortality	0	0	0
35	perlite	NH000	Dead - ***Pythium***	0	0	0
8	perlite	NH000	Dead - ***Pythium***	0	0	0
15	sand	GN107		3	2	67
22	sand	GN107		1	0	0
25	sand	GN107	Dead-cutback mortality	0	0	0
4	sand	GN107	Substandard plant ?	0	0	0
10	sand	GN156		1	0	0
28	sand	GN156		2	2	100
29	sand	GN156		3	3	100
32	sand	GN156		2	0	0
1	sand	NH000	Dead - ***Pythium***	0	0	0
13	sand	NH000	Dead-cutback mortality	0	0	0
14	sand	NH000	Dead - ***Pythium***	0	0	0
34	sand	NH000	Dead-cutback mortality	0	0	0

C.9 **Rooting results for trough system** (sorted by substrate)

Plot No.	Treatment	Clone No	Status	Cuttings placed
1	Gravel	GN107		2
2	Gravel	GN156		4
3	Gravel	NH000		1
4	Gravel	NH000		2
5	Gravel	NH000		4
6	Gravel	GN156		9
7	Gravel	GN107		3
8	Gravel	GN107		5
9	Gravel	NH000	Dead - ***Pythium***	0
10	Gravel	NH000	Dead - ***Pythium***	0
11	Gravel	GN107	Dead - ***Pythium***	0
12	Gravel	GN156		5
13	Gravel	GN156		6
14	Gravel	GN107		3
15	Gravel	GN156		4
16	Sand	NH000		2
17	Sand	GN156		6
18	Sand	GN107		6
19	Sand	GN107		7
20	Sand	GN156		5
21	Sand	GN107		8
22	Sand	NH000		6
23	Sand	NH000		3
24	Sand	GN107		6
25	Sand	GN156		4
26	Sand	GN107		3
27	Sand	NH000		6
28	Sand	GN156		6
29	Sand	GN156		4
30	Sand	NH000	Dead - ***Pythium***	0
31	Perlite	GN156		3
32	Perlite	NH000		2
33	Perlite	GN156		6
34	Perlite	GN156		8
35	Perlite	NH000		11
36	Perlite	GN156		7
37	Perlite	GN107		7
38	Perlite	NH000		8
39	Perlite	GN107		5
40	Perlite	NH000		3
41	Perlite	NH000		9
42	Perlite	GN156		2
43	Perlite	GN107		2
44	Perlite	GN107		2

Plot No.	Treatment	Clone No	Status	Cuttings placed
45	Perlite	GN107		1
46	NFT	NH000		2
47	NFT	GN156		5
48	NFT	GN156		9
49	NFT	GN156		4
50	NFT	NH000		10
51	NFT	GN107		10
52	NFT	GN107	Dead - ***Pythium***	0
53	NFT	GN156		5
54	NFT	NH000		6
55	NFT	GN107	Substandard plant?	0
56	NFT	GN107		6
57	NFT	NH000		10
58	NFT	GN156		6
59	NFT	GN107		3
60	NFT	GN107		19
61	NFT	NH000		10
62	NFT	GN156		12
63	NFT	GN107	Dead - ***Pythium***	0
64	NFT	GN156		8
65	NFT	NH000		1
66	NFT	NH000	Substandard plant?	0

C.10 Linear regression output: effect of ambient temperature on substrate

*** Degrees of freedom ***
Sums of squares: 19
Sums of products: 18
Correlations: 18

*** Sums of squares and products ***
Gut_max_ 1 89.75
Air_temp 2 23.00 264.00
1 2
*** Means ***
Gut_max_ 1 18.75
Air_temp 2 15.00

*** Number of units used ***
20

*** Correlation matrix ***
Gut_max_ 1 1.000
Air_temp 2 0.149 1.000
1 2

***** Regression Analysis *****
Response variate: Gut_max_
Fitted terms: Constant, Air_temp

*** Summary of analysis ***

	d.f.	s.s.	M.s.	V.r.	F pr.
Regression	1	2.00	2.004	0.41	0.530
Residual	18	87.75	4.875		
Total	19	89.75	4.724		
Change	-1	-2.00	2.004	0.41	0.530

Residual variance exceeds variance of Y variate
Standard error of observations is estimated to be 2.21
* MESSAGE: The following units have large standardized residuals:

13	-2.67
18	2.29

*** Estimates of regression coefficients ***

	estimate	s.e.	t18)	t pr.
Constant	17.44	2.10	8.32	<.001
Air_temp	0.087	0.136	0.64	0.530

*** Degrees of freedom ***
Sums of squares: 19
Sums of products: 18
Correlations: 18

*** Sums of squares and products ***

Gut_min_	1	236.8	
Air_temp	2	173.0	264.0
		1	2

*** Means ***

Gut_min_	1	12.60
Air_temp	2	15.00

*** Number of units used ***
20
*** Correlation matrix ***

Gut_min_	1	1.000	
Air_temp	2	0.692	1.000
		1	2

***** Regression Analysis *****
Response variate: Gut_min_
Fitted terms: Constant, Air_temp

*** Summary of analysis ***

	d.f.	s.s.	M.s.	v.r.	F pr.
Regression	1	113.4	113.367	16.53	<.001
Residual	18	123.4	6.857		
Total	19	236.8	12.463		
Change	-1	-113.4	113.367	16.53	<.001

Percentage variance accounted for 45.0
Standard error of observations is estimated to be 2.62

*** Estimates of regression coefficients ***

	estimate	s.e.	t(18)	t pr.
Constant	2.77	2.49	1.11	0.280
Air_temp	0.655	0.161	4.07	<.001

*** Degrees of freedom ***
Sums of squares: 19
Sums of products: 18
Correlations: 18

*** Sums of squares and products ***

Pot_max_	1	80.55	
Air_temp	2	42.00	264.00
		1	2

*** Means ***

Pot_max_	1	18.35
Air_temp	2	15.00

*** Correlation matrix ***

Pot_max_	1	1.000	
Air_temp	2	0.288	1.000
		1	2

***** Regression Analysis *****
Response variate: Pot_max_
Fitted terms: Constant, Air_temp

*** Summary of analysis ***

	d.f.	s.s.	M.s.	v.r.	F pr.
Regression	1	6.68	6.682	1.63	0.218
Residual	18	73.87	4.104		
Total	19	80.55	4.239		
Change	-1	-6.68	6.682	1.63	0.218

Percentage variance accounted for 3.2
Standard error of observations is estimated to be 2.03
* MESSAGE: The following units have large standardized residuals:

13	-2.71

*** Estimates of regression coefficients ***

	estimate	s.e.	t(18)	t pr.
Constant	15.96	1.92	8.30	<.001
Air_temp	0.159	0.125	1.28	0.218

*** Sums of squares and products ***

Pot_min_	1	272.9	
Air_temp	2	170.0	264.0
		1	2

*** Means ***

Pot_min_	1	12.05
Air_temp	2	15.00

*** Number of units used ***
20

*** Correlation matrix ***

Pot_min_	1	1.000	
Air_temp	2	0.633	1.000
		1	2

**** Regression Analysis *****
Response variate: Pot_min_
Fitted terms: Constant, Air_temp

*** Summary of analysis ***

	d.f.	s.s.	M.s.	v.r.	F pr.
Regression	1	109.5	109.470	12.05	0.003
Residual	18	163.5	9.082		
Total	19	272.9	14.366		
Change	-1	-109.5	109.470	12.05	0.003

Percentage variance accounted for 36.8
Standard error of observations is estimated to be 3.01

*** Estimates of regression coefficients ***

	estimate	s.e.	t(18)	t pr.
Constant	2.39	2.86	0.84	0.415
Air_temp	0.644	0.185	3.47	0.003

***** Regression Analysis *****

Response variate: Pot_min_
Fitted terms: Constant, Air_temp

*** Summary of analysis ***

	d.f.	s.s.	M.s.	V.r.	F pr.
Regression	1	109.5	109.470	12.05	0.003
Residual	18	163.5	9.082		
Total	19	272.9	14.366		
Change	-1	-109.5	109.470	12.05	0.003

Percentage variance accounted for 36.8
Standard error of observations is estimated to be 3.01

*** Estimates of regression coefficients ***

	estimate	s.e.	t(18)	t pr.
Constant	2.39	2.86	0.84	0.415
Air_temp	0.644	0.185	3.47	0.003

APPENDIX D

RESPONSE OF CLONAL EUCALYPT HEDGE PLANTS TO THREE NUTRIENT SOLUTIONS IN A RECIRCULATING HYDROPONIC SYSTEM

D.1 Randomisation of experiment

D.1.1 Trial layout - **Mondi Orange fertiliser** (planted 22/6/2000)

Plot no.	NFT	Gravel	Perlite	Sand
1	GN107	NH000	NH000	GN107
2	GN107	GN156	GN107	NH000
3	GN156	GN107	GN107	NH000
4	GN156	NH000	GN156	GN107
5	NH000	GN156	GN156	GN156
6	NH000	GN107	GN107	GN156
7	GN156	GN156	NH000	NH000
8	GN107	NH000	NH000	GN107
9	NH000	GN107	GN107	GN107
10	NH000	GN156	GN156	GN156
11	GN107	GN107	NH000	NH000
12	GN156	GN107	GN156	NH000
13	GN156	GN156	GN156	GN107
14	NH000	NH000	GN107	GN156
15	NH000	NH000	NH000	GN107
16	GN156	GN107	GN107	NH000
17	GN107	GN107	NH000	GN156
18	NH000	NH000	GN107	GN156
19	NH000	GN156	NH000	GN107
20	GN107	NH000	GN107	GN156
21	GN156	GN156	GN156	NH000
22	GN156	GN107	NH000	GN156
23	GN107	NH000	GN156	GN107
24	GN107	GN156	GN156	NH000

D.1.1.1 Ramet dimensions at planting, Mondi Orange fertiliser

Plot no.	NFT	Shoot length cm	Shoot diam. mm	No. of leaves	Gravel	Shoot length cm	Shoot diam. mm	No. of leaves
1	GN107	10.70	1.20	8	NH000	12.00	2.00	10
2	GN107	11.40	2.20	7	GN156	15.00	2.10	8
3	GN156	14.00	2.20	10	GN107	14.00	1.40	8
4	GN156	12.00	1.80	11	NH000	7.50	2.10	8
5	NH000	15.00	2.00	9	GN156	14.00	2.70	7
6	NH000	10.50	2.00	8	GN107	7.00	1.30	6
7	GN156	13.50	1.80	8	GN156	12.50	2.10	8
8	GN107	9.00	1.30	6	NH000	9.50	1.70	7
9	NH000	15.00	2.50	9	GN107	10.50	1.90	8
10	NH000	11.00	1.90	6	GN156	14.50	2.00	8
11	GN107	14.50	2.10	10	GN107	7.00	1.20	6
12	GN156	14.00	2.10	10	GN107	10.00	1.90	8
13	GN156	12.50	2.20	10	GN156	12.50	1.80	6
14	NH000	17.00	1.80	10	NH000	11.50	2.10	8
15	NH000	18.50	2.00	10	NH000	12.00	2.10	6
16	GN156	17.00	2.00	10	GN107	10.00	1.90	8
17	GN107	13.50	2.00	8	GN107	15.00	2.00	8
18	NH000	10.50	1.70	6	NH000	11.00	1.80	6
19	NH000	6.00	1.80	4	GN156	10.00	2.00	7
20	GN107	11.00	1.80	8	NH000	14.00	2.50	9
21	GN156	16.00	2.10	10	GN156	12.70	1.90	8
22	GN156	12.50	2.00	8	GN107	12.50	1.90	8
23	GN107	12.00	2.00	8	NH000	6.20	1.90	6
24	GN107	11.00	2.00	6	GN156	12.50	2.60	8
MEAN		**12.84**	**1.94**	**8.33**		**11.39**	**1.95**	**7.50**

D.1.1.1 Ramet dimensions at planting, Mondi Orange fertiliser

Plot no.	Perlite	Shoot length cm	Shoot diam. mm	No. Of leaves	Sand	Shoot length cm	Shoot diam. mm	No. Of leaves
1	NH000	16.3	2.1	10	GN107	8.2	2.2	7
2	GN107	8.5	1.5	6	NH000	11.4	2	8
3	GN107	13.5	2.1	8	NH000	15.8	2	10
4	GN156	10.5	1.8	8	GN107	11	1.8	10
5	GN156	17.3	2.8	10	GN156	15.7	2	9
6	GN107	12.5	2.3	8	GN156	14.5	2	10
7	NH000	17.1	2.2	10	NH000	13	2.2	10
8	NH000	8.8	1.3	8	GN107	13.2	2.2	11
9	GN107	11.2	2	8	GN107	10.3	1.8	8
10	GN156	15.5	2.1	10	GN156	11	2	11
11	NH000	9	1.6	6	NH000	13.5	2.2	8
12	GN156	11	2	10	NH000	13.5	1.9	8
13	GN156	11.3	1.6	7	GN107	10.5	2	8
14	GN107	10.9	1.8	8	GN156	10.2	1.5	9
15	NH000	16.2	2.1	8	GN107	12.5	2.5	9
16	GN107	12.5	2	8	NH000	10	1.5	7
17	NH000	11.3	1.9	6	GN156	13.7	2	9
18	GN107	11.3	2	8	GN156	12	2	12
19	NH000	13	2.2	8	GN107	12.4	2	8
20	GN107	10.5	1.6	8	GN156	9.3	2	10
21	GN156	10.3	2.1	6	NH000	9.2	1.5	6
22	NH000	14	2.1	8	GN156	10.5	2.2	8
23	GN156	13	1.9	8	GN107	13.5	2	8
24	GN156	13.2	1.8	10	NH000	7.5	1.8	8
MEAN		**12.45**	**1.95**	**8.13**		**11.77**	**1.97**	**8.83**

D.1.2 **Trial layout, Mondi MM fertiliser** (planted 22/6/2000)

Plot no.	NFT	Gravel	Perlite	Sand
1	GN156	NH000	GN156	NH000
2	GN107	GN156	GN107	GN107
3	GN107	NH000	GN107	GN107
4	GN107	GN107	GN156	GN107
5	GN156	GN107	NH000	GN156
6	NH000	GN156	GN156	NH000
7	GN107	GN107	GN107	GN156
8	NH000	NH000	NH000	GN107
9	NH000	GN156	NH000	GN156
10	GN156	GN156	NH000	GN156
11	GN107	NH000	GN107	NH000
12	NH000	GN156	GN107	GN107
13	GN107	GN107	GN156	GN156
14	NH000	GN107	GN156	GN156
15	GN156	NH000	NH000	NH000
16	GN156	GN107	GN156	GN107
17	NH000	GN107	GN156	NH000
18	GN156	GN156	NH000	GN107
19	GN107	GN156	GN107	NH000
20	NH000	NH000	NH000	NH000
21	GN107	GN156	GN107	GN107
22	NH000	NH000	GN107	GN156
23	GN156	NH000	GN156	GN156
24	GN156	GN107	NH000	NH000

D.1.2.1 Ramet dimensions at planting, Mondi MM4 fertiliser

Plot no.	NFT	Shoot length cm	Shoot diam. mm	No. of leaves	Gravel	Shoot length cm	Shoot diam. mm	No. of leaves
1	GN156	16.00	2.10	10	NH000	10.00	2.00	8
2	GN107	13.50	2.10	8	GN156	9.40	1.50	8
3	GN107	12.00	2.00	10	NH000	10.50	1.80	6
4	GN107	14.50	1.50	8	GN107	9.50	1.70	6
5	GN156	15.50	2.10	10	GN107	10.70	1.90	8
6	NH000	18.00	2.10	8	GN156	10.00	2.00	8
7	GN107	10.00	1.90	8	GN107	10.80	1.70	6
8	NH000	12.50	2.00	8	NH000	9.00	1.40	5
9	NH000	15.00	2.40	10	GN156	13.50	2.10	10
10	GN156	12.50	1.80	8	GN156	11.00	2.10	10
11	GN107	9.00	1.20	8	NH000	13.00	2.10	9
12	NH000	13.80	3.00	8	GN156	13.00	2.20	10
13	GN107	15.50	2.30	10	GN107	12.00	2.10	8
14	NH000	10.00	2.10	8	GN107	9.00	1.80	5
15	GN156	9.50	1.30	5	NH000	16.00	2.10	8
16	GN156	10.00	1.80	6	GN107	14.00	2.20	12
17	NH000	12.00	2.10	4	GN107	9.00	1.30	6
18	GN156	9.50	2.00	6	GN156	12.00	2.00	10
19	GN107	8.00	1.10	6	GN156	12.70	2.20	8
20	NH000	14.50	1.90	8	NH000	11.30	1.60	7
21	GN107	11.50	2.10	8	GN156	15.00	2.10	8
22	NH000	13.20	2.10	6	NH000	12.50	2.00	6
23	GN156	11.00	1.80	6	NH000	12.00	2.00	8
24	GN156	15.50	2.10	9	GN107	10.00	1.30	6
MEAN		**12.60**	**1.95**	**7.75**		**11.50**	**1.88**	**7.75**

D.1.2.1 Ramet dimensions at planting, Mondi MM4 fertiliser

Plot no.	Perlite	Shoot length cm	Shoot diam. mm	No. of leaves	Sand	Shoot length cm	Shoot diam. mm	No. of leaves
1	GN156	15.00	2.20	8	NH000	8.50	2.10	8
2	GN107	10.00	1.60	8	GN107	8.70	1.80	8
3	GN107	9.50	2.10	8	GN107	11.00	2.10	8
4	GN156	11.50	2.00	10	GN107	10.00	1.70	6
5	NH000	13.00	1.50	8	GN156	14.00	2.00	10
6	GN156	10.00	1.70	8	NH000	14.50	2.00	10
7	GN107	15.00	2.00	8	GN156	14.50	2.00	10
8	NH000	19.00	2.50	10	GN107	10.00	2.00	8
9	NH000	12.50	2.00	8	GN156	9.00	1.20	8
10	NH000	11.50	2.10	6	GN156	13.50	2.00	8
11	GN107	10.00	1.80	8	NH000	18.00	2.20	9
12	GN107	7.50	2.00	8	GN107	8.50	2.00	6
13	GN156	14.00	1.50	8	GN156	11.70	1.70	6
14	GN156	13.50	2.00	8	GN156	14.50	2.00	8
15	NH000	14.00	1.90	8	NH000	13.00	2.00	8
16	GN156	12.00	2.00	8	GN107	10.20	2.80	6
17	GN156	10.30	2.00	8	NH000	12.00	2.00	6
18	NH000	6.50	1.20	6	GN107	11.90	1.80	6
19	GN107	11.50	2.10	9	NH000	14.00	1.90	8
20	NH000	9.00	1.50	4	NH000	9.50	1.50	6
21	GN107	10.00	2.00	8	GN107	11.00	2.00	10
22	GN107	11.70	1.80	6	GN156	14.00	1.80	8
23	GN156	9.30	1.30	8	GN156	9.10	1.60	6
24	NH000	16.00	1.20	10	NH000	16.40	2.10	8
MEAN		**11.76**	**1.83**	**7.88**		**11.98**	**1.93**	**7.71**

D.1.3 Trial Layout, Hydroponica® fertiliser (planted 22/6/2000)

Plot no.	NFT	Gravel	Perlite	Sand
1	GN156	GN107	GN156	NH000
2	GN156	GN107	NH000	GN107
3	NH000	GN156	GN107	GN156
4	GN156	GN156	GN156	NH000
5	GN107	GN107	NH000	GN156
6	NH000	NH000	GN156	GN107
7	GN107	NH000	NH000	GN156
8	NH000	GN107	GN107	GN156
9	GN107	GN156	GN107	NH000
10	NH000	NH000	NH000	GN107
11	GN107	NH000	NH000	GN156
12	GN156	GN156	GN156	GN107
13	GN156	GN156	GN107	NH000
14	NH000	GN107	GN107	NH000
15	GN156	NH000	GN156	GN156
16	GN107	NH000	GN107	GN107
17	NH000	GN107	NH000	GN107
18	NH000	GN156	GN156	NH000
19	GN107	NH000	GN107	GN156
20	GN156	NH000	GN156	GN156
21	GN156	GN156	NH000	GN107
22	GN107	GN156	GN107	NH000
23	GN107	GN107	GN156	NH000
24	NH000	GN107	NH000	GN107

D.1.3.1 Ramet dimensions at planting, Hydroponica® fertiliser

Plot no.	NFT	Shoot length cm	Shoot diam. mm	No. of leaves	Gravel	Shoot length cm	Shoot diam. mm	No. of leaves
1	GN156	13.50	2.20	8	GN107	11.50	1.90	10
2	GN156	13.20	2.20	9	GN107	9.40	1.70	6
3	NH000	19.00	2.10	10	GN156	8.60	1.60	8
4	GN156	11.40	2.10	12	GN156	11.10	1.70	8
5	GN107	9.10	2.00	9	GN107	15.40	2.30	11
6	NH000	11.60	1.90	8	NH000	11.10	1.60	9
7	GN107	6.50	1.60	5	NH000	14.00	2.20	8
8	NH000	11.50	2.70	8	GN107	9.00	2.30	6
9	GN107	9.50	1.70	9	GN156	13.00	2.10	9
10	NH000	8.80	1.80	6	NH000	15.70	2.40	9
11	GN107	11.00	2.20	8	NH000	11.30	1.60	7
12	GN156	14.70	2.20	10	GN156	13.90	2.00	8
13	GN156	10.90	1.80	8	GN156	14.80	1.80	8
14	NH000	12.00	1.90	8	GN107	11.40	2.20	8
15	GN156	12.60	2.10	10	NH000	16.20	2.10	9
16	GN107	7.20	1.40	6	NH000	16.00	2.30	10
17	NH000	17.60	2.20	8	GN107	11.30	1.90	8
18	NH000	8.80	1.90	8	GN156	11.40	2.20	7
19	GN107	11.60	1.80	6	NH000	14.20	2.30	8
20	GN156	8.50	1.50	8	NH000	15.40	2.30	10
21	GN156	11.70	1.70	8	GN156	9.80	2.10	7
22	GN107	8.70	1.50	5	GN156	11.00	1.70	8
23	GN107	8.80	1.50	6	GN107	5.10	1.40	8
24	NH000	11.50	2.30	6	GN107	11.20	2.00	8
MEAN		**11.24**	**1.93**	**7.88**		**12.16**	**1.99**	**8.25**

D.1.3.1 Ramet dimensions at planting, Hydroponica® fertiliser

Plot no.	Perlite	Shoot length cm	Shoot diam. mm	No. of leaves	Sand	Shoot length cm	Shoot diam. mm	No. of leaves
1	GN156	10.50	1.50	7	NH000	19.00	2.50	11
2	NH000	13.00	1.70	6	GN107	13.50	2.00	8
3	GN107	12.00	1.70	10	GN156	13.00	2.10	8
4	GN156	8.00	1.50	7	NH000	13.00	1.30	8
5	NH000	13.50	2.00	9	GN156	10.40	2.10	8
6	GN156	9.50	1.60	8	GN107	11.50	2.00	9
7	NH000	12.50	2.00	8	GN156	13.00	1.80	8
8	GN107	6.50	1.10	6	GN156	11.50	2.20	8
9	GN107	12.00	2.00	8	NH000	14.00	2.00	7
10	NH000	16.50	2.10	8	GN107	11.00	1.60	7
11	NH000	13.00	2.00	10	GN156	12.00	2.00	9
12	GN156	13.00	2.30	10	GN107	7.00	2.00	6
13	GN107	11.00	2.20	9	NH000	12.00	2.00	8
14	GN107	12.50	2.10	7	NH000	15.50	2.00	10
15	GN156	13.50	2.10	10	GN156	14.50	2.00	9
16	GN107	14.50	2.00	11	GN107	8.50	2.00	8
17	NH000	15.50	2.20	9	GN107	11.50	1.90	8
18	GN156	8.50	1.50	10	NH000	12.50	2.20	8
19	GN107	9.00	2.10	9	GN156	14.00	2.20	11
20	GN156	13.00	2.00	12	GN156	15.00	2.10	10
21	NH000	8.00	1.10	6	GN107	8.50	1.60	5
22	GN107	9.50	2.00	9	NH000	20.00	2.00	11
23	GN156	8.50	1.10	9	NH000	14.50	2.00	9
24	NH000	16.00	1.80	8	GN107	10.00	2.20	7
MEAN		**11.65**	**1.82**	**8.58**		**12.73**	**1.99**	**8.38**

D.2 Measured light intensity for experiment (Lux)

Date	Position	Mondi Orange	Mondi MM4	Hydroponica®
28/6/2000	1	2597	2963	3097
	2	4447	4937	4943
	3	3467	3603	3653
29/6/2000	1	2503	2643	2877
	2	4180	4607	4550
	3	3103	3307	3360
30/6/2000	1	2653	3113	3380
	2	4213	4767	4920
	3	3170	3567	3680
3/7/2000	1	2623	2903	3130
	2	4240	4840	4853
	3	3317	3453	3383
4/7/2000	1	2473	2737	3037
	2	4210	4597	4857
	3	3187	3330	3453
5/7/2000	1	2697	3243	3110
	2	4403	4897	4753
	3	3657	3527	3487
6/7/2000	1	2737	3137	3567
	2	4307	4837	5170
	3	3250	3797	3720
7/7/2000	1	2593	3087	3030
	2	4237	4563	4736
	3	3230	3360	3423
10/7/2000	1	2567	3120	3423
	2	4193	4713	4900
	3	3123	3383	3503
11/7/2000	1	2537	2730	2820
	2	4233	4417	4623
	3	3230	3333	3327
12/7/2000	1	2673	2547	2847
	2	4340	4623	4470
	3	3187	3130	3050
MEAN		**3381**	**3691**	**3792**

D.3 Cuttings yield

D.3.1 Cuttings placed, **Mondi Orange** (cutting date: 6/10/2000) rooting hormone - Seradix 1

Clone ID No.	Treatment	Cuttings placed	Mean cuttings n=8
GN 107	sand	33	4.13
NH 00	sand	26	3.25
GN 156	sand	30	3.75
NH 00	perlite	35	4.38
GN 107	perlite	34	4.25
GN 156	perlite	41	5.13
NH 00	gravel	32	4.00
GN 156	gravel	41	5.13
GN 107	gravel	31	3.88
GN 107	NFT	33	4.13
GN 156	NFT	48	6.00
NH 00	NFT	59	7.38
Total		**443**	**4.62**

D.3.2 Cuttings placed, **Hydroponica®** (cutting date: 6/10/2000) rooting hormone - Seradix 1

Clone ID No.	Treatment	Cuttings placed	Mean cuttings n=8
GN 156	NFT	51	6.38
GN 107	NFT	49	6.13
NH 00	NFT	51	6.38
GN 107	gravel	27	3.38
GN 156	gravel	52	6.50
NH 00	gravel	59	7.38
GN 156	perlite	43	5.38
NH 00	perlite	49	6.13
GN 107	perlite	28	3.50
NH 00	sand	66	8.25
GN 107	sand	47	5.88
GN 156	sand	73	9.13
Total		**595**	**6.20**

D.3.3 Cuttings placed, **Mondi MM4** (cutting date: 6/10/2000) rooting hormone - Seradix 1

Clone ID No.	Treatment	Cuttings placed	Mean cuttings n=8
GN 156	NFT	76	9.50
GN 107	NFT	88	11.00
NH 00	NFT	98	12.25
NH 00	gravel	57	7.13
GN 156	gravel	59	7.38
GN 107	gravel	40	5.00
GN 156	perlite	46	5.75
GN 107	perlite	46	5.75
NH 00	perlite	52	6.50
NH 00	sand	50	6.25
GN 107	sand	40	5.00
GN 156	sand	40	5.00
Total		**692**	**7.21**

APPENDIX E

RESPONSE OF CLONAL EUCALYPT HEDGE PLANTS TO SEVEN NUTRIENT SOLUTIONS IN A RECIRCULATING HYDROPONIC SYSTEM

E.1 Destructive harvest 28/11/2001 (harvest 1)

Fertiliser	Substrate	Clone ID	Placed Crop 1	Rooted Crop1	Root % Crop 1	RCD (mm)	Length (cm)
Hydroponica	Gravel	NH0000	26	6	23.08	2.14	5.42
Hydroponica	Gravel	GN156	24	6	25.00	1.94	4.58
Hydroponica	Gravel	GN107	72	27	37.50	1.92	4.83
Hydroponica	Sand	NH0000	36	5	13.89	1.43	3.90
Hydroponica	Sand	GN156	17	0	0.00	0.00	0.00
Hydroponica	Sand	GN107	60	2	3.33	1.85	4.25
Hydroponica	Perlite	NH0000	7	1	14.29	1.43	4.00
Hydroponica	Perlite	GN156	26	1	3.85	1.78	7.00
Hydroponica	Perlite	GN107	43	5	11.63	1.41	4.60
Hydroponica	NFT	NH0000	43	1	2.33	1.08	3.50
Hydroponica	NFT	GN156	6	0	0.00	0.00	0.00
Hydroponica	NFT	GN107	9	0	0.00	0.00	0.00
M & S	Gravel	NH0000	11	2	18.18	1.61	3.50
M & S	Gravel	GN156	16	0	0.00	0.00	0.00
M & S	Gravel	GN107	53	7	13.21	1.68	4.64
M & S	Sand	NH0000	15	10	66.67	1.61	5.40
M & S	Sand	GN156	9	0	0.00	0.00	0.00
M & S	Sand	GN107	52	1	1.92	1.22	4.00
M & S	Perlite	NH0000	30	10	33.33	1.29	4.00
M & S	Perlite	GN156	13	1	7.69	1.60	5.50
M & S	Perlite	GN107	12	2	16.67	1.82	5.50
M & S	NFT	NH0000	14	3	21.43	1.47	4.50
M & S	NFT	GN156	32	2	6.25	1.47	4.50
M & S	NFT	GN107	18	0	0.00	0.00	0.00
Natgro	Gravel	NH0000	52	4	7.69	1.24	4.00
Natgro	Gravel	GN156	25	2	8.00	2.00	3.50
Natgro	Gravel	GN107	43	3	6.98	1.62	5.67
Natgro	Sand	NH0000	11	0	0.00	0.00	0.00
Natgro	Sand	GN156	10	1	10.00	1.47	2.00
Natgro	Sand	GN107	62	5	8.06	1.21	4.80
Natgro	Perlite	NH0000	13	3	23.08	1.73	5.33
Natgro	Perlite	GN156	62	11	17.74	1.70	4.05
Natgro	Perlite	GN107	30	3	10.00	2.05	4.83
Natgro	NFT	NH0000	20	5	25.00	2.02	4.60
Natgro	NFT	GN156	40	8	20.00	1.39	3.81
Natgro	NFT	GN107	14	2	14.29	1.81	5.00
Hoaglands	Gravel	NH0000	29	11	37.93	1.34	3.95
Hoaglands	Gravel	GN156	23	5	21.74	1.60	4.20
Hoaglands	Gravel	GN107	45	5	11.11	1.56	3.80
Hoaglands	Sand	NH0000	9	3	33.33	1.44	4.67
Hoaglands	Sand	GN156	6	2	33.33	1.45	4.25
Hoaglands	Sand	GN107	29	7	24.14	1.38	4.43
Hoaglands	Perlite	NH0000	10	2	20.00	1.63	4.25
Hoaglands	Perlite	GN156	19	5	26.32	1.91	5.60
Hoaglands	Perlite	GN107	26	11	42.31	1.65	4.09
Hoaglands	NFT	NH0000	46	9	19.57	1.65	4.06

Fertiliser	Substrate	Clone ID	Placed Crop 1	Rooted Crop1	Root % Crop 1	RCD (mm)	Length (cm)
Hoaglands	NFT	GN156	40	12	30.00	1.40	3.04
Hoaglands	NFT	GN107	27	1	3.70	1.40	2.50
Orange	Gravel	NH0000	45	7	15.56	1.62	4.00
Orange	Gravel	GN156	41	2	4.88	2.05	4.50
Orange	Gravel	GN107	51	3	5.88	1.57	4.50
Orange	Sand	NH0000	42	8	19.05	1.86	4.50
Orange	Sand	GN156	48	11	22.92	1.33	3.41
Orange	Sand	GN107	34	4	11.76	1.66	4.13
Orange	Perlite	NH0000	18	2	11.11	1.73	4.00
Orange	Perlite	GN156	31	7	22.58	1.64	4.86
Orange	Perlite	GN107	71	6	8.45	1.51	4.42
Orange	NFT	NH0000	40	9	22.50	1.55	3.33
Orange	NFT	GN156	23	1	4.35	1.60	5.00
Orange	NFT	GN107	66	0	0.00	0.00	0.00
MM4	Gravel	NH0000	18	2	11.11	1.88	3.50
MM4	Gravel	GN156	29	1	3.45	2.04	5.00
MM4	Gravel	GN107	47	4	8.51	1.52	4.38
MM4	Sand	NH0000	9	1	11.11	1.99	4.00
MM4	Sand	GN156	24	3	12.50	1.48	4.33
MM4	Sand	GN107	63	2	3.17	1.96	3.50
MM4	Perlite	NH0000	18	1	5.56	1.55	5.00
MM4	Perlite	GN156	43	8	18.60	1.55	4.31
MM4	Perlite	GN107	37	0	0.00	0.00	0.00
MM4	NFT	NH0000	50	11	22.00	1.57	3.82
MM4	NFT	GN156	15	4	26.67	1.68	3.75
MM4	NFT	GN107	22	1	4.55	1.87	4.00
Hydrofeed	Gravel	NH0000	35	15	42.86	1.48	3.53
Hydrofeed	Gravel	GN156	27	4	14.81	1.62	4.88
Hydrofeed	Gravel	GN107	41	8	19.51	2.00	4.94
Hydrofeed	Sand	NH0000	13	1	7.69	2.17	6.00
Hydrofeed	Sand	GN156	67	19	28.36	1.39	4.34
Hydrofeed	Sand	GN107	32	3	9.38	1.68	5.50
Hydrofeed	Perlite	NH0000	48	9	18.75	1.54	4.83
Hydrofeed	Perlite	GN156	35	10	28.57	1.70	4.75
Hydrofeed	Perlite	GN107	31	2	6.45	1.56	4.00
Hydrofeed	NFT	NH0000	43	15	34.88	1.67	4.37
Hydrofeed	NFT	GN156	29	4	13.79	1.75	5.00
Hydrofeed	NFT	GN107	62	7	11.29	1.45	3.50

E. 2 **Destructive harvest 04/02/2002** (harvest 2)

Fertiliser	Substrate	Clone ID	Placed Crop 2	Rooted Crop 2	Root % Crop 2	RCD (mm)	Length (cm)
Hydroponica	Gravel	NH0000	102	18	17.60	1.44	4.06
Hydroponica	Gravel	GN156	58	6	10.30	1.20	4.94
Hydroponica	Gravel	GN107	23	2	8.70	1.23	4.75
Hydroponica	Sand	NH0000	43	9	20.90	1.66	5.11
Hydroponica	Sand	GN156	38	5	13.20	1.46	4.00
Hydroponica	Sand	GN107	51	0	0.00	0.00	0.00
Hydroponica	Perlite	NH0000	69	4	5.80	1.45	4.88
Hydroponica	Perlite	GN156	39	1	2.60	1.21	4.00
Hydroponica	Perlite	GN107	27	8	29.60	1.37	4.69
Hydroponica	NFT	NH0000	61	2	3.30	1.29	5.50
Hydroponica	NFT	GN156	59	6	10.20	1.13	5.83
Hydroponica	NFT	GN107	27	2	7.40	1.29	4.00
M & S	Gravel	NH0000	55	14	25.50	1.49	4.54
M & S	Gravel	GN156	39	0	0.00	0.00	0.00
M & S	Gravel	GN107	37	2	5.40	1.11	4.25
M & S	Sand	NH0000	60	20	33.30	1.54	4.78
M & S	Sand	GN156	65	11	16.90	1.31	4.86
M & S	Sand	GN107	35	1	2.90	1.04	5.00
M & S	Perlite	NH0000	51	21	41.20	1.47	5.43
M & S	Perlite	GN156	47	4	8.50	1.83	4.75
M & S	Perlite	GN107	56	3	5.40	1.62	3.17
M & S	NFT	NH0000	63	6	9.50	1.49	5.67
M & S	NFT	GN156	88	3	3.40	1.88	3.17
M & S	NFT	GN107	63	4	6.30	1.72	4.25
Natgro	Gravel	NH0000	46	9	19.60	1.28	5.44
Natgro	Gravel	GN156	30	4	13.30	1.20	4.00
Natgro	Gravel	GN107	41	5	12.20	1.67	5.60
Natgro	Sand	NH0000	28	7	25.00	1.67	5.29
Natgro	Sand	GN156	24	0	0.00	0.00	0.00
Natgro	Sand	GN107	27	3	11.10	1.62	5.17
Natgro	Perlite	NH0000	36	7	19.40	1.49	5.29
Natgro	Perlite	GN156	24	1	4.20	2.00	3.50
Natgro	Perlite	GN107	71	4	5.60	2.31	3.88
Natgro	NFT	NH0000	59	11	18.60	1.68	5.41
Natgro	NFT	GN156	19	0	0.00	0.00	0.00
Natgro	NFT	GN107	47	12	25.50	1.56	4.67
Hoaglands	Gravel	NH0000	65	17	26.20	1.37	4.44
Hoaglands	Gravel	GN156	73	7	9.60	1.53	5.50
Hoaglands	Gravel	GN107	42	6	14.30	1.90	4.83
Hoaglands	Sand	NH0000	58	6	10.30	1.23	6.25
Hoaglands	Sand	GN156	20	2	10.00	1.36	5.50
Hoaglands	Sand	GN107	32	1	3.10	1.72	6.00
Hoaglands	Perlite	NH0000	54	27	50.00	1.79	4.91
Hoaglands	Perlite	GN156	48	2	4.20	1.26	5.00
Hoaglands	Perlite	GN107	35	0	0.00	0.00	0.00
Hoaglands	NFT	NH0000	48	18	37.50	1.68	5.14
Hoaglands	NFT	GN156	38	19	50.00	1.18	5.00
Hoaglands	NFT	GN107	38	4	10.50	1.28	5.37
Orange	Gravel	NH0000	34	5	14.70	1.57	5.70
Orange	Gravel	GN156	27	7	25.90	1.50	4.21
Orange	Gravel	GN107	54	2	3.70	1.65	3.00
Orange	Sand	NH0000	45	7	15.60	1.58	4.86
Orange	Sand	GN156	26	4	15.40	1.85	4.50
Orange	Sand	GN107	19	0	0.00	0.00	0.00
Orange	Perlite	NH0000	80	10	12.50	2.10	3.70
Orange	Perlite	GN156	77	1	1.30	1.42	4.50
Orange	Perlite	GN107	51	1	2.00	1.26	6.00

Fertiliser	Substrate	Clone ID	Placed Crop 2	Rooted Crop 2	Root % Crop 2	RCD (mm)	Length (cm)
Orange	NFT	NH0000	30	1	3.30	1.63	5.00
Orange	NFT	GN156	29	0	0.00	0.00	0.00
Orange	NFT	GN107	23	3	13.00	1.27	4.50
MM4	Gravel	NH0000	72	23	31.90	1.54	3.50
MM4	Gravel	GN156	56	6	10.70	1.36	6.08
MM4	Gravel	GN107	38	6	15.80	1.72	3.75
MM4	Sand	NH0000	49	6	12.20	1.34	4.00
MM4	Sand	GN156	42	1	2.40	1.30	3.00
MM4	Sand	GN107	49	2	4.10	1.75	4.00
MM4	Perlite	NH0000	59	16	27.10	1.53	4.72
MM4	Perlite	GN156	48	0	0.00	0.00	0.00
MM4	Perlite	GN107	44	1	2.30	1.56	5.00
MM4	NFT	NH0000	80	24	30.00	1.25	3.60
MM4	NFT	GN156	33	2	6.10	1.15	4.00
MM4	NFT	GN107	24	0	0.00	0.00	0.00
Hydrofeed	Gravel	NH0000	48	1	2.10	2.04	5.00
Hydrofeed	Gravel	GN156	59	0	0.00	0.00	0.00
Hydrofeed	Gravel	GN107	30	3	10.00	1.76	4.17
Hydrofeed	Sand	NH0000	34	2	5.90	1.33	5.00
Hydrofeed	Sand	GN156	41	5	12.20	1.72	5.30
Hydrofeed	Sand	GN107	23	1	4.30	1.93	3.00
Hydrofeed	Perlite	NH0000	48	11	22.90	2.00	4.64
Hydrofeed	Perlite	GN156	30	1	3.30	1.27	4.00
Hydrofeed	Perlite	GN107	40	1	2.50	2.28	4.00
Hydrofeed	NFT	NH0000	66	15	22.70	1.67	4.40
Hydrofeed	NFT	GN156	57	6	10.50	1.51	5.47
Hydrofeed	NFT	GN107	53	0	0.00	0.00	0.00

E.3 Destructive harvest 28/03/2002 (harvest 3)

Fertiliser	Substrate	Clone ID	Placed Crop 3	Rooted Crop 3	Root % Crop 3	RCD (mm)	Length (cm)
Hydroponica	Gravel	NH000	49	24	48.98	1.85	5.23
Hydroponica	Gravel	GN107	35	27	77.14	2.20	5.17
Hydroponica	Gravel	GN156	29	20	68.97	1.85	5.40
Hydroponica	Sand	NH000	23	0	0.00	0.00	0.00
Hydroponica	Sand	GN107	26	15	57.69	1.93	5.60
Hydroponica	Sand	GN156	47	32	68.09	2.12	5.22
Hydroponica	Perlite	NH000	44	21	47.73	2.51	5.74
Hydroponica	Perlite	GN107	49	24	48.98	2.25	5.29
Hydroponica	Perlite	GN156	69	31	44.93	1.41	4.61
Hydroponica	NFT	NH000	26	19	73.08	2.25	5.97
Hydroponica	NFT	GN107	20	13	65.00	1.57	5.73
Hydroponica	NFT	GN156	32	11	34.38	2.31	5.36
M & S	Gravel	NH000	39	33	84.62	1.82	5.45
M & S	Gravel	GN107	37	9	24.32	1.75	5.06
M & S	Gravel	GN156	16	10	62.50	1.55	5.35
M & S	Sand	NH000	20	7	35.00	2.52	5.50
M & S	Sand	GN107	27	11	40.74	1.97	5.00
M & S	Sand	GN156	27	10	37.04	1.79	5.20
M & S	Perlite	NH000	45	35	77.78	1.58	5.43
M & S	Perlite	GN107	37	18	48.65	1.73	5.53
M & S	Perlite	GN156	20	12	60.00	1.85	5.29
M & S	NFT	NH000	23	13	56.52	1.86	5.50
M & S	NFT	GN107	16	9	56.25	1.57	5.06
M & S	NFT	GN156	37	22	59.46	1.56	4.48
Natgro	Gravel	NH000	19	8	42.11	2.21	4.75
Natgro	Gravel	GN107	16	10	62.50	1.86	5.35
Natgro	Gravel	GN156	8	4	50.00	1.86	5.49
Natgro	Sand	NH000	18	15	83.33	2.09	5.60
Natgro	Sand	GN107	20	10	50.00	1.85	5.70
Natgro	Sand	GN156	25	9	36.00	1.84	5.72
Natgro	Perlite	NH000	21	10	47.62	1.92	4.85
Natgro	Perlite	GN107	19	15	78.95	1.97	5.60
Natgro	Perlite	GN156	18	12	66.67	2.19	5.83
Natgro	NFT	NH000	22	13	59.09	1.66	4.73
Natgro	NFT	GN107	15	8	53.33	1.98	4.56
Natgro	NFT	GN156	26	17	65.38	1.67	5.44
Hoaglands	Gravel	NH000	47	23	48.94	1.85	4.48
Hoaglands	Gravel	GN107	31	4	12.90	2.13	5.38
Hoaglands	Gravel	GN156	71	16	22.54	1.59	4.44
Hoaglands	Sand	NH000	23	16	69.57	1.87	5.41
Hoaglands	Sand	GN107	30	4	13.33	1.25	3.75
Hoaglands	Sand	GN156	29	9	31.03	1.35	3.83
Hoaglands	Perlite	NH000	40	34	85.00	2.09	5.44
Hoaglands	Perlite	GN107	43	17	39.53	1.77	5.26
Hoaglands	Perlite	GN156	30	14	46.67	2.13	5.18
Hoaglands	NFT	NH000	34	18	52.94	1.26	4.39
Hoaglands	NFT	GN107	25	6	24.00	1.98	4.42
Hoaglands	NFT	GN156	31	8	25.81	1.57	4.56
Orange	Gravel	NH000	27	18	66.67	1.87	5.61
Orange	Gravel	GN107	40	12	30.00	1.79	5.00
Orange	Gravel	GN156	55	15	27.27	1.99	4.70

Fertiliser	Substrate	Clone ID	Placed Crop 3	Rooted Crop 3	Root % Crop 3	RCD (mm)	Length (cm)
Orange	Sand	NH000	41	27	65.85	1.94	5.81
Orange	Sand	GN107	29	21	72.41	1.58	5.55
Orange	Sand	GN156	43	19	44.19	1.91	4.84
Orange	Perlite	NH000	31	20	64.52	2.28	4.93
Orange	Perlite	GN107	62	20	32.26	1.86	4.58
Orange	Perlite	GN156	29	12	41.38	1.74	5.38
Orange	NFT	NH000	18	13	72.22	1.56	5.19
Orange	NFT	GN107	14	1	7.14	1.51	3.50
Orange	NFT	GN156	13	5	38.46	1.65	6.00
MM4	Gravel	NH000	32	28	87.50	2.07	5.75
MM4	Gravel	GN107	17	9	52.94	1.93	5.44
MM4	Gravel	GN156	37	16	43.24	2.06	5.50
MM4	Sand	NH000	56	28	50.00	1.95	4.46
MM4	Sand	GN107	14	2	14.29	1.81	4.75
MM4	Sand	GN156	34	25	73.53	2.01	5.76
MM4	Perlite	NH000	38	20	52.63	2.16	5.43
MM4	Perlite	GN107	28	15	53.57	2.00	5.27
MM4	Perlite	GN156	24	9	37.50	1.80	5.56
MM4	NFT	NH000	46	41	89.13	1.77	5.35
MM4	NFT	GN107	37	18	48.65	1.82	4.89
MM4	NFT	GN156	29	7	24.14	1.54	5.43
Hydrofeed	Gravel	NH000	106	47	44.34	1.82	4.19
Hydrofeed	Gravel	GN107	40	18	45.00	1.99	5.58
Hydrofeed	Gravel	GN156	51	17	33.33	2.50	5.06
Hydrofeed	Sand	NH000	120	36	30.00	1.99	4.32
Hydrofeed	Sand	GN107	99	22	22.22	1.57	4.16
Hydrofeed	Sand	GN156	86	41	47.67	2.21	5.04
Hydrofeed	Perlite	NH000	29	19	65.52	2.45	6.03
Hydrofeed	Perlite	GN107	25	7	28.00	1.83	5.93
Hydrofeed	Perlite	GN156	40	20	50.00	2.42	5.88
Hydrofeed	NFT	NH000	40	16	40.00	2.45	5.77
Hydrofeed	NFT	GN107	66	12	18.18	1.84	4.33
Hydrofeed	NFT	GN156	36	8	22.22	2.01	5.81

E.4 Mortality count due to severe cut back (04/02/2002)

Fertiliser	Medium	Plant No.	Clone	Comment
Hydroponica	Gravel	5	NH000	?
		17	NH000	Dead
		26	NH000	Dead
	Sand	3	GN156	?
		10	NH000	?
		13	NH000	Replaced
	Perlite	5	NH000	?
		6	GN156	Dead
		12	GN107	Dead
		19	GN156	Dead
	NFT	3	NH000	?
		10	GN156	?
		14	NH000	Dead
		18	NH000	?
		21	NH000	Replaced
		24	NH000	?
M & S	Gravel	2	NH000	Dead
		6	NH000	Dead
		11	NH000	Dead
		19	GN156	Dead
		21	NH000	?
		26	GN107	?
	Sand	1	GN107	Dead
		8	GN156	Dead
		9	NH000	Dead
		12	NH000	?
		14	NH000	Dead
		26	NH000	Replaced
		27	GN156	Dead
	Perlite	1	GN156	Dead
		5	GN107	?
		6	GN156	Dead
		7	NH000	Dead
		9	GN107	Dead
		10	NH000	Dead
		19	NH000	Dead
		28	NH000	Dead
	NFT	5	NH000	Dead
		18	GN156	?
		25	GN156	Dead
		27	NH000	Dead
		30	NH000	Dead
Natgro	Perlite	14	GN107	Dead
Hoagland	Perlite	4	NH000	Dead
		10	GN107	Dead
		13	GN156	Dead
		15	NH000	Dead
		17	GN107	Dead
	NFT	3	GN156	?
		13	GN107	Dead
		14	GN156	Dead
		25	GN107	Dead
Orange	Gravel	18	NH000	Dead
		20	NH000	?
	Sand	1	GN156	Dead
		8	NH000	Dead
	Perlite	2	NH000	Dead
		22	NH000	Dead

Fertiliser	Medium	Plant No.	Clone	Comment
MM4	Gravel	11	NH000	Dead
		18	NH000	Dead
		20	NH000	Dead
		21	GN156	Dead
		25	NH000	Dead
	Sand	11	NH000	?
		12	GN156	Dead
		24	NH000	Dead
	Perlite	3	NH000	Dead
		7	NH000	Dead
		9	GN156	Dead
		15	NH000	Dead
		20	NH000	Dead
		22	NH000	Dead
	NFT	17	NH000	?
Hydrofeed	Gravel	1	GN107	Dead
		6	NH000	Dead
		10	NH000	Dead
		12	GN107	Dead
		13	NH000	?
		14	GN107	?
		17	NH000	Dead
		18	GN156	Dead
		21	NH000	Dead
		22	GN156	?
		23	GN107	?
		24	NH000	?
		25	NH000	Dead
		27	GN107	?
		28	GN156	Dead
		29	NH000	Dead
	Sand	1	NH000	Dead
		4	NH000	Dead
		7	NH000	Dead
		8	GN156	?
		10	GN107	?
		15	NH000	Dead
		16	NH000	Dead
		21	GN156	Dead
		30	NH000	Dead
	Perlite	6	NH000	Dead
		12	GN156	Dead
		13	NH000	Dead
		16	NH000	Dead
		24	GN156	Dead
	NFT	2	NH000	Dead
		7	GN156	Dead
		14	GN107	Dead
		17	NH000	?
		20	GN156	Dead
		28	GN156	Dead
Hydroponica	Gravel	6	GN156	Dead
	Sand	1	GN107	Dead
		7	GN156	Dead
		10	GN156	Replaced
		11	NH000	Replaced
		12	GN156	Dead